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TESIS DOCTORAL

DETERMINACIÓN Y MODELADO DE LOS PATRONES ESPACIALES DE LA TRUCHA COMÚN (*Salmo trutta* Linnaeus, 1758) EN LA CUENCA DEL RÍO DEVA-CARES: EL ROL DE LA CONECTIVIDAD Y DEL NICHO

PhD THESIS

DETERMINATION AND MODELING OF SPATIAL PATTERNS OF BROWN TROUT (*Salmo trutta* Linnaeus, 1758) IN THE DEVA-CARES CATCHMENT: THE ROLE OF CONNECTIVITY AND THE NICHE

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A mis padres
A mi abuela
A mi hermano

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Capítulo I. Introducción y antecedentes de la investigación

1.1 Antecedentes y motivación

Los ecosistemas acuáticos de agua dulce contienen alrededor del 0.01% del agua mundial y cubren el 0.8% de la superficie terrestre (Dudgeon et al., 2006). No obstante, los ecosistemas acuáticos de agua dulce presentan una gran riqueza de especies en relación a su área, conteniendo cerca del 6% de todas las especies descritas (Dudgeon et al., 2006), de las cuales alrededor de 12700 especies son peces (44% de la diversidad global de peces y 9.1% del total de especies de agua dulce; Lévêque et al., 2008; E. Balian et al., 2010). Sin embargo, diferentes impactos antrópicos están amenazando la biodiversidad acuática y los recursos de agua dulce para la sociedad humana (Vörösmarty et al., 2010). Las amenazas para la diversidad acuática se agrupan en cinco categorías principalmente: sobreexplotación, contaminación del agua, alteración de caudales, destrucción y degradación del hábitat e invasión de especies exóticas (Dudgeon et al., 2006). Su combinación e interacción ha causado un declive de las poblaciones y un aumento del riesgo de extinción a nivel mundial (Dudgeon et al., 2006) afectando la composición, estructura y función de las comunidades acuáticas.

Los peces de agua dulce son uno de los grupos más vulnerables a los impactos antrópicos. Estimas globales indican que alrededor del 25% de las especies de peces de agua dulce evaluadas están en peligro de extinción (Vié et al., 2009). Estos niveles son más altos en Europa debido a su alto nivel de endemismos, dónde el 37% de las especies de peces evaluadas están en peligro de extinción (Freyhof & Wright, 2011). Concretamente, la Península Ibérica es considerada como un hotspot de especies de peces de agua dulce dentro de Europa (Reyjol et al., 2007) y está caracterizada por

presentar un elevado número de especies endémicas. La especies piscícolas Ibéricas también se encuentran dentro de las más amenazadas a nivel Europeo, por lo que necesitan de una especial atención para su gestión y conservación debido a su alta vulnerabilidad a los impactos antrópicos.

La vulnerabilidad de una determinada especie piscícola a diferentes amenazas puede estar influenciada por su biología, capacidad de dispersión, requerimientos del hábitat o características históricas de la vida de la especie (Angermeier, 1995; Reynolds et al., 2005). Además, la vulnerabilidad de las comunidades o incluso del ecosistema (incluyendo el funcionamiento ecosistémico) son dependientes de las condiciones físicas, pero también de las interdependencias biológicas que se establecen a nivel de comunidad (e.g. la perdida de un depredador a nivel local puede inducir extinciones secundarias; Borrvall & Ebenman, 2006). En este sentido, los estudios enfocados en especies clave (Paine, 1966) son importantes debido a que la variabilidad de las densidades de la especie tiene un gran impacto en los procesos del ecosistema y las comunidades biológicas a través de la depredación, competición o ingenieros del ecosistema (ver Cottee-Jones & Whittaker, 2012 para más información sobre especies clave). Una adecuada gestión y conservación de los ecosistemas de agua dulce es necesaria para mejorar nuestro conocimiento sobre la distribución y los factores que controlan los patrones espaciales o temporales de estas especies clave.

Una de las especies piscícolas de agua dulce considerada como especie clave en previos estudios (e.g. Tzilkowski, 2005) y que requiere especial atención por su importancia ecológica, social y económica en la Península Ibérica es la trucha común (*Salmo trutta* Linnaeus, 1758), especie en la que se centra esta Tesis Doctoral.

1.2 Trucha común

La trucha común es un salmónido naturalmente distribuido en toda la región este del Atlántico y norte del Mediterráneo (Eurasia y norte de África; Elliott, 1989c), aunque ha sido introducida por la actividad humana en todos los continentes (excepto en la región Antártica; B. Jonsson & Jonsson, 2011). Es una especie polimórfica con una elevada diversidad morfológica e historia evolutiva que varía intra e interpoblaciones (Elliott, 1989b; Milner et al., 2003) con poblaciones lacustres, anádromas y residentes.

La Península Ibérica representa el límite sur de la distribución natural de la especie, dónde dominan las poblaciones residentes. Las poblaciones lacustres están ausentes (Alonso et al., 2012) y las poblaciones anádromas solo están presentes en el Norte desde la latitud 42ºN (Hamilton et al., 1989). Las dinámicas espaciales y temporales de la especie están afectadas por factores denso-dependientes (Milner et al., 2003) y denso-independientes (Alonso et al., 2012), dependiendo del ciclo de vida y de las condiciones ambientales (Elliott, 1989b), aunque ambos tipos pueden operar simultaneamente. La selección espacial del nicho está estructurada por tamaños (Heggenes et al., 1999; Ayllón et al., 2010) debido a que las diferentes clases de edad tienen preferencias y requerimientos energéticos distintos. Las poblaciones están compuestas de individuos móviles y estacionarios (e. g. Bridcut & Giller, 1993), siendo la fracción móvil menos abundante en proporción (Young et al., 2010). En relación a las diferentes clases de edad, la dispersión de los alevines es limitada (Vatland & Caudron, 2015) mientras que los adultos son los que presentan las mayores tasas de dispersión (Olsson & Greenberg, 2004).

La trucha común tiene una gran importancia ecológica, económica y social, siendo uno de los depredadores principales en los ecosistemas fluviales (Jensen et al., 2008; Sánchez-Hernández, 2016) y una de las principales especies de pesca deportiva (Almodovar & Nicola, 1998). A pesar de su importancia, las poblaciones Ibéricas han disminuido en los últimos años por factores como la introgresión genética, la sobrepesca, las especies invasoras, la contaminación, el cambio global, la fragmentación y la pérdida del hábitat (Doadrio, 2002; Almodovar et al., 2012; Maceda-Veiga, 2013).

En esta Tesis, las poblaciones de trucha común serán analizadas desde un punto de vista del nicho en la red fluvial incluyendo la dispersión y la conectividad, además de considerar la importancia de la estructura metapoblacional sobre estas poblaciones.

1.3 El concepto de nicho

El nicho es un concepto central en los campos de la ecología y la evolución introducido por Grinnell (1917) y redefinido por Elton (1927), pero la formalización del concepto de nicho fue dada por Hutchinson (1959, 1978) diferenciando dos tipos: (1) el nicho fundamental que es el hipervolúmen de n-dimensiones que ocupa una especie en la ausencia de competidores y (2) el nicho realizado que es la porción del nicho

fundamental que ocupa una especie en presencia de competidores. Cada dimensión representa una variable biótica o abiótica importante para la persistencia de la especie.

La distribución espacial de las especies y sus abundancias están con frecuencia relacionas con la amplitud y posición de sus nichos (Jorgensen & Fath, 2014). Estudios previos han mostrado la importancia del nicho en las poblaciones piscícolas fluviales (Heggenes et al., 1999; Pörtner et al., 2010) y en recientes décadas, se han usado Modelos de Distribución de Especies (MDS) para relacionar las condiciones ambientales con la distribución espacial (Leathwick et al., 2005; González-Ferreras et al., 2016).

Contraria a la teoría del nicho, Hubbell (2001, 2005) propuso la teoría neutral, asumiendo que todas las especies son funcionalmente equivalentes y que su distribución está afectada principalmente por procesos estocásticos, destacando la importancia de los procesos de dispersión. Esta teoría también se ha demostrado que es importante para las especies piscícolas (Muneepeerakul et al., 2008). La teoría neutral y la teoría del nicho se consideran dos extremos de un continuo (Gravel et al., 2006) y ambos enfoques son importantes para las comunidades fluviales, aunque puede ser difícil separar la influencia relativa de cada proceso. Por lo tanto, los patrones espaciales pueden ser determinados por el nicho y por la dispersión (Jorgensen & Fath, 2014). El concepto de dispersión se comenta en una sección posterior.

En relación al nicho y la especie objeto de estudio en esta Tesis Doctoral, la distribución de las poblaciones de trucha común están influenciadas por su hábitat incluyendo tanto factores bióticos como abióticos. Por un lado, los factores abióticos más importantes son la temperatura, el caudal, la profundidad, el sustrato, la velocidad del agua y el refugio, mostrando también diferencias entre clases de edad (ver Heggenes et al., 1999; Armstrong et al., 2003 para una descripción detallada). Por otro lado, los factores abióticos más importantes son los factores denso-dependientes intra y entre-cohortes (Cattanéo et al., 2002; Parra et al., 2012; Ayllón et al., 2013) y las enfermedades (Schager et al., 2007).

1.4 El nicho en redes fluviales

Los ecosistemas fluviales poseen una naturaleza cuatridimensional (longitudinal, lateral, vertical y temporal; Ward, 1989) y están caracterizados por una estructura dendrítica y

anidada jerárquicamente y por su conectividad y direccionalidad dónde las redes fluviales son elementos clave del paisaje que integran dinámicas ecológicas, hidrológicas y geomorfológicas (Rodriguez-Iturbe et al., 2009). La clasificación jerárquica de hábitats de Frissell et al. (1986) es la clasificación más usada que reconoce la estructura espacial jerárquica de los ecosistemas fluviales. En esta clasificación, los sistemas fluviales incluyen todas las corrientes de aguas superficiales de una cuenca, y están organizados jerárquicamente incorporando sucesivamente en los niveles anteriores segmentos, tramos, pool/riffle y microhábitats. Esta estructura jerárquica diferencia los ecosistemas fluviales del resto de los ecosistemas acuáticos y terrestres (Fullerton et al., 2010). La disposición espacial y la estructura jerárquica de los hábitats influyen en la distribución y en los patrones espaciales de las poblaciones y las interacciones de las comunidades (Campbell Grant et al., 2007). Además, diferentes actividades humanas pueden alterar estos patrones (Branco et al., 2012).

Los estudios de investigación y gestión llevados a cabo en los ecosistemas fluviales se deben de realizar a escalas apropiadas que respondan a la cuestión de interés (Fausch et al., 2002). La mayoría de estudios realizados enfocados en las especies piscícolas se han llevado a cabo a nivel local (i.e. en unos pocos metros dentro de un segmento o tramo de la red fluvial), aunque en algunos casos los resultados obtenidos pueden conllevar a conclusiones erróneas si no se ha considerado la escala que incluye la historia vital de la especie (Fausch et al., 2002). Por ejemplo, algunos estudios han mostrado que el hábitat usado por los peces puede ser más grande que la escala usada para su gestión (Cooper & Mangel, 1999). Por lo tanto, la evaluación a nivel de tramo o segmento *per se* no permite comprender efectos más amplios a nivel de una red fluvial competa y los estudios a nivel local pueden mostrar resultados diferentes cuando se considera toda la cuenca.

El paradigma “Riverscape” (Fausch et al., 2002) evidencia estas diferencias entre la escala a la que se toman las medidas y las respuestas biológicas que ocurren en el ecosistema proponiendo una visión continua de la red fluvial. Así pues, “Riverscape” se define como “un mosaico de hábitats fluviales que esta espacialmente estructurado y jerárquicamente organizado a lo largo de múltiples escalas” (Davis et al., 2018). Varios estudios previos han conducido a alcanzar este paradigma (Hawkes, 1975; Vannote et al., 1980; Rice et al., 2001; Poole, 2002; Benda et al., 2004). Por lo tanto, los paradigmas tradicionales se han ido reemplazando por modelos y enfoques que tienen

en cuenta la escala, jerarquía, complejidad y heterogeneidad de los ecosistemas fluviales destacando la importancia de la red fluvial y sus propiedades como el tamaño de hábitats o la conectividad (Kuemmerlen et al., 2019). Actualmente hay varios estudios enfocados en redes fluviales usando diferentes técnicas de modelado y con diferentes objetivos (e.g. Mari et al., 2014; González-Ferreras et al., 2016; Álvarez-Cabria et al., 2017; Rodríguez-Castillo et al., 2018).

Concretamente, el estudio de González-Ferreras et al. (2016) es considerado como un estudio preliminar que sentó las bases de esta Tesis Doctoral. En este estudio preliminar, se usaron MDS para determinar la distribución potencial de seis especies piscícolas, incluida la trucha común, en varias cuencas del norte de España, basándose exclusivamente en su nicho. Varias razones hicieron de este estudio previo un paso clave en el desarrollo de la Tesis Doctoral.

Primero, solo datos piscícolas provenientes de diferentes confederaciones y agencias del agua se usaron en los MDS. Las evidencias encontradas por González-Ferreras et al. (2016) en el diseño de los datos de entrenamiento de los modelos conllevaron a los autores a descartar estos datos en futuros estudios y recopilar nuevos datos piscícolas con una resolución y diseño espacial apropiado a nivel de red fluvial. Estos autores mostraron que un dataset de entrenamiento no balanceado, como el caso de la trucha común debido a su alto porcentaje de ocurrencia para el área de estudio, obtenía un nivel predictivo bajo independientemente del modelo o método de evaluación utilizado. La inclusión de datos de ausencias creando un dataset más equilibrado mejoraba la capacidad predictiva del modelo, poniendo de manifiesto que para analizar los patrones espaciales de una especie generalista a nivel de cuenca, se necesitan más datos que incluyan información no solo de presencias, sino también de ausencias. Para cubrir este déficit de información en las bases de datos existentes, se diseñó un muestreo específico que cubriese toda la red fluvial para obtener datos adecuados que representasen toda la variabilidad espacial de la especie para esta Tesis Doctoral. Además, el estudio preliminar se basó en datos de presencia-ausencia, pero dada la importancia potencial de la variabilidad en la densidad de las poblaciones de la trucha común por su impacto potencial sobre los procesos ecosistémicos y las comunidades biológicas, hemos considerado más apropiado analizar variaciones espaciales de densidad en lugar de limitar los análisis a la distribución de presencias-ausencias.

Segundo, durante el desarrollo del estudio preliminar, los autores observaron que para determinar la distribución actual en lugar de la distribución potencial, las características del nicho pueden no ser suficientes, dada la importancia de la conectividad y la dispersión y su influencia en la estructura de las poblaciones. Por esta razón, hemos decidido incluir estos procesos en los análisis siguientes para determinar los patrones espaciales de la trucha común a nivel de cuenca y analizar qué factores son los más importantes.

Finalmente, con el propósito de analizar los patrones espaciales a nivel de cuenca en más detalle y con la ausencia de perturbaciones importantes en la calidad del agua, hemos decidido estudiar una de las cuencas incluidas en el estudio de González-Ferreras et al. (2016), la cuenca del río Deva-Cares. Esta cuenca incluye varias barreras longitudinales, tanto antrópicas como naturales, pero otras presiones están casi ausentes, representando un área idónea para analizar el rol de la conectividad y del nicho.

1.5 Conectividad y dispersión en redes fluviales

Los ecosistemas fluviales están controlados por la conectividad hidrológica definida como "la transferencia de materia, energía u organismos por el agua dentro de los elementos del ciclo hidrológico" (Pringle, 2001). Aunque la conectividad comprende interacciones a lo largo de las cuatro dimensiones de los ecosistemas fluviales (Ward, 1989) y todas son importantes para las especies fluviales, la conectividad longitudinal es la más importante para las especies piscícolas debido a los movimientos y migraciones por la red fluvial para completar su ciclo de vida entre los diferentes parches de hábitats (Segurado et al., 2015). Por lo tanto, la estructura de la red fluvial, la historia vital y/o los rasgos de dispersión de las especies pueden afectar la conectividad entre las poblaciones acuáticas (J. M. Hughes et al., 2009). Por un lado, la estructura de la red fluvial puede afectar a las poblaciones al influir en el movimiento de los individuos o la transferencia de flujos desde aguas arriba hacia aguas abajo o viceversa (Lowe et al., 2006). Por otro lado, la historia vital y la dispersión son elementos clave para entender muchos patrones y procesos poblacionales (Lidicker & Stenseth, 1992) incluyendo poblaciones acuáticas que se dispersan estrictamente por la red fluvial (como la trucha común) y poblaciones que se pueden dispersar por el medio terrestre en alguna etapa de su ciclo de vida (e.g. algunas etapas de los insectos; Chaput-Bardy et al., 2017).

Aunque las redes fluviales están naturalmente fragmentadas (e.g. cascadas o rápidos), las acciones antrópicas han fragmentado aún más estos hábitats (e.g. presas o azudes). Además de los cambios ambientales producidos por la fragmentación en las redes fluviales, la alteración de la conectividad afecta principalmente a la calidad, cantidad y accesibilidad de los parches de hábitat y a la dispersión de las poblaciones piscícolas (Larinier, 2000) por el efecto barrera producido. Por lo tanto, la fragmentación y la pérdida del hábitat pueden producir varios efectos en las poblaciones piscícolas como la extinción de poblaciones aisladas (Morita & Yamamoto, 2002), divergencia genética (M. M. Hansen et al., 2014) o dispersión asimétrica (Junker et al., 2012) entre otras.

Los impactos a nivel local producidos por las barreras han sido ampliamente investigados (e.g. Lessard & Hayes, 2003; Greathouse et al., 2006; Katano et al., 2006; Gardner et al., 2013). Sin embargo, estos estudios pueden proveer información insuficiente para entender las consecuencias a escalas más amplias (Campbell Grant et al., 2007). Hasta la fecha, existen pocos estudios empíricos que aborden como los cambios de la conectividad pueden afectar a diferentes atributos del ecosistema a escala de red fluvial (pero véase Ziv et al., 2012; Van Looy et al., 2014). En este sentido y debido a la influencia de la conectividad en las poblaciones piscícolas, es importante incorporar esta información a nivel de cuenca para estudiar los patrones espaciales de las poblaciones piscícolas.

1.6 Dinámicas metapoblacionales

El término de metapoblación fue introducido por Levins (1969) sugiriendo una población compuesta de poblaciones locales que se extinguen y se recolonizan y cuyas dinámicas garantizan la persistencia de la población global. El modelo metapoblacional de Levins asume que todas las poblaciones y parches de hábitat son iguales expresando una descripción poco realista de las metapoblaciones naturales debido a su simplicidad. Consecuentemente, varias modificaciones al modelo de Levins han incorporado diferencias en las poblaciones locales y en los parches de hábitat (i.e. tamaño y calidad; Gilpin et al., 1991). De acuerdo a Hansky et al. (1997) una metapoblacion se define como “un conjunto de poblaciones locales dentro de un área más grande, dónde es posible la migración desde una población local a al menos alguno de los otros parches”.

Tres condiciones definen una metapoblación: 1) las poblaciones locales habitan parches de hábitat discretos, 2) la dinámica de los parches ocupados no es sincrónica y 3) existen eventos de dispersión entre los parches de hábitats (Rieman & Dunham, 2000; Schtickzelle & Quinn, 2007). Harrison (1991) categorizó la estructura metapoblacional en cuatro tipos: clásica, mainland-island y source sink, patchy y non-equilibrium indicando que la magnitud de la dispersión entre parches y la variabilidad en la calidad y el tamaño de los parches de hábitat determinan el tipo de metapoblación.

Fagan (2002) mostró como los modelos metapoblacionales clásicos no capturan la estructura dendrítica y jerárquica de los sistemas fluviales y estudios posteriores han descrito los efectos espaciales que la conectividad ejerce sobre la demografía y genética de poblaciones (e.g. Fagan, 2002; Muneepeerakul et al., 2007; Labonne et al., 2008; Muneepeerakul et al., 2008; Fullerton et al., 2016). La teoría de metapoblaciones se ha mostrado relevante para las especies de salmónidos, pero a pesar de su importancia e interés, los estudios empíricos al respecto son escasos (pero véase Rieman & Dunham, 2000; Falke & Fausch, 2010). En el caso de la trucha común, los estudios llevados a cabo muestran resultados diversos como estructura metapoblacional (mainland-island o source sink; Østergaard et al., 2003) o asilamiento por distancia (Wright, 1943) mostrando una población distribuida en continuo con una disminución en la similaridad genética al aumentar la distancia geográfica (Griffiths et al., 2009).

Determinar los patrones espaciales de las poblaciones de trucha común a nivel de cuenca y analizar la influencia del nicho y la conectividad son cuestiones clave para identificar la estructura de la población. Debido a que estas poblaciones se encuentran amenazadas por varios impactos antrópicos, principalmente fragmentación y pérdida del hábitat, que pueden alterar la estructura y la persistencia de las poblaciones, entender que factores son los que determinan la variabilidad espacial de las poblaciones de la trucha común es esencial para llevar a cabo acciones de conservación y gestión adecuadas.

1.7 Objetivos de la Tesis

El objetivo general de la Tesis Doctoral es determinar los patrones espaciales de la trucha común en la red fluvial de la cuenca del río Deva-Cares y analizar los diferentes roles que la conectividad y el nicho tienen en las determinación de la variabilidad espacial de las poblaciones de la trucha común. Entender cuáles son los principales factores que determinan los patrones espaciales de la especie para toda la red fluvial es un importante avance en el campo de la ecología fluvial y también es esencial para preservar y mejorar el estado de conservación de las poblaciones. Por lo tanto, los resultados obtenidos en esta Tesis, tendrán un alto valor tanto desde un punto de vista científico como en el diseño de estrategias de conservación y gestión eficientes a escala de cuenca.

Los objetivos específicos de esta Tesis se centran en los siguientes aspectos (Figura 1):

- Determinar el hábitat potencial disponible para la trucha común considerando la extensión de la red fluvial permanente en la cuenca del río Deva-Cares desarrollando una estrategia de modelado para: (1) estimar la ocurrencia y extensión de los segmentos permanentes y temporales en una red fluvial completa a escala de cuenca y (2) determinar las variables principales en la determinación de su distribución (Capítulo III).

- Explorar y cuantificar que variables ambientales a diferentes escalas espaciales (cuenca, segmento y tramo) tienen un rol más importante para determinar la densidad de cada clase de edad de la trucha común e investigar si la distancia hidrológica y Euclidea y la presencia de barreras infranqueables son importantes para determinar los patrones espaciales de la trucha común (Capítulo IV).

- Desarrollar un modelo metapoblacional para estimar los patrones espaciales medios de las densidades de la trucha común para cada clase de edad basado en la topología de la red fluvial, la conectividad y las dinámicas poblacionales y explorar los efectos de la conectividad y la dispersión en los patrones espaciales medios de las densidades de la trucha común (Capítulo V).

- Investigar las consecuencias genéticas de la alteración de la conectividad en la población de la trucha común en la red fluvial de la cuenca del río Deva-Cares y

describir los patrones de la estructura poblacional explorando: (1) la variabilidad genética de la trucha común en la cuenca, (2) la diferenciación genética y la estructura de la población, (3) las tasas de migración históricas y los tamaños efectivos de población y (4) las causas de la diferenciación genética y características del paisaje (Capítulo VI).

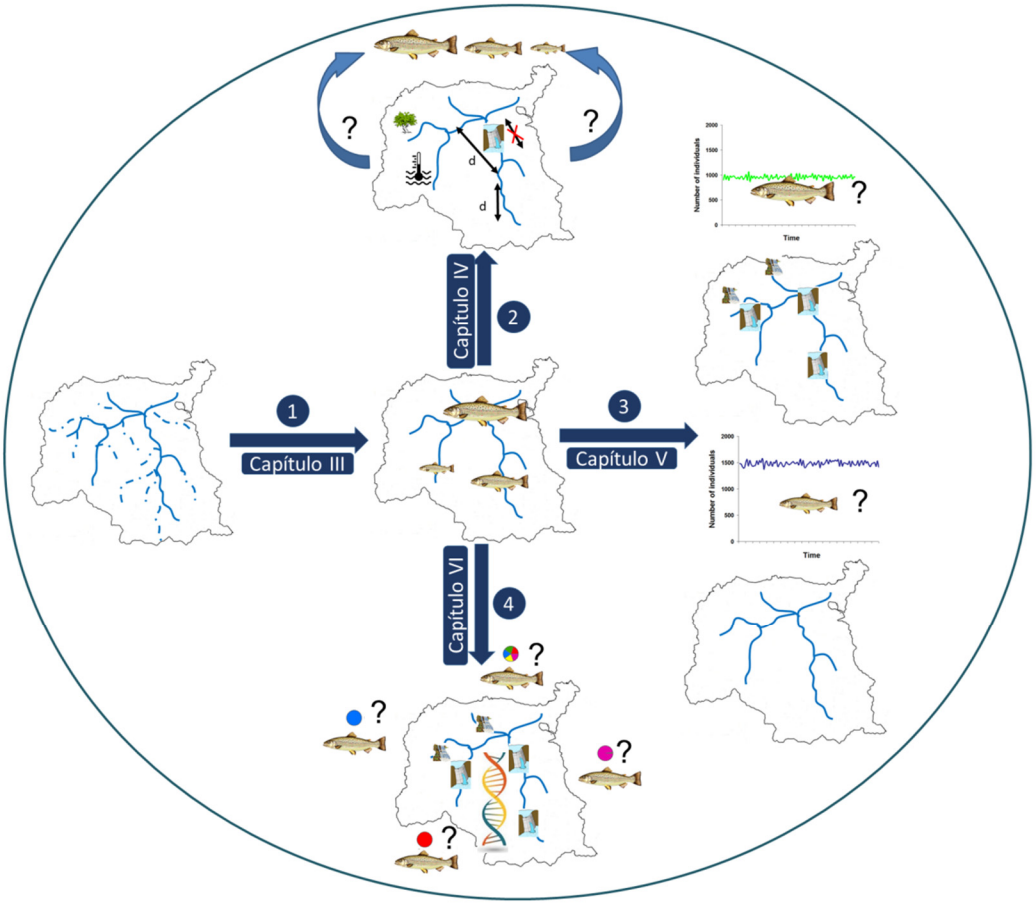

Figura 1. Diagrama conceptual que integra los diferentes enfoques en relación a los objetivos específicos desarrollados en los capítulos centrales de la Tesis Doctoral.

Capítulo II. Área de estudio

2.1 Área de estudio

El área de estudio es la cuenca del río Deva-Cares (1200 km^2), situada en el norte de España (Figura 2).

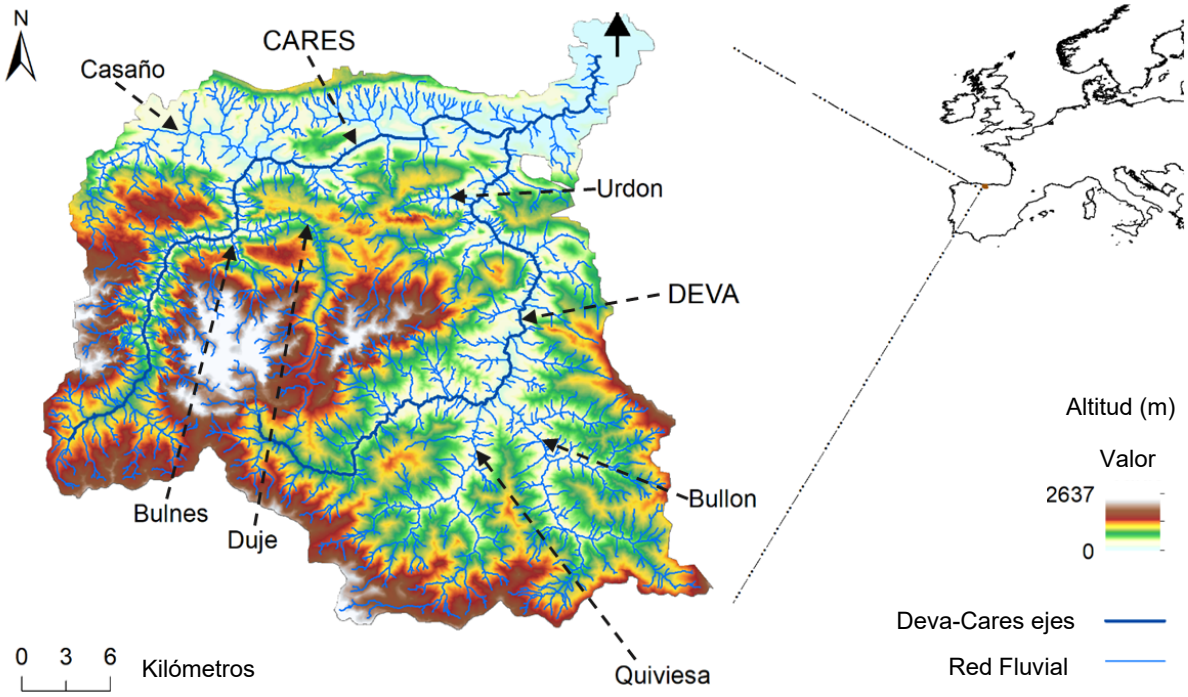

Figura 2. Localización de la cuenca del río Deva-Cares, principales tributarios de los ríos Deva y Cares y representación del modelo digital de elevación.

2.2 Geología, geomorfología y suelos

El área de estudio se localiza en la Cordillera Cantábrica, la cual se ha originado por la sucesiva acción de la orogenia Herciniana (periodo Carbonífero) y Alpina (periodo Terciario). Desde un punto de vista geológico, la cuenca está situada entre la región Vasco-Cantábrica (caracterizada por sedimentos Mesozoicos) y la región Astur-Galaica (caracterizada por la ausencia o rareza de sedimentos Mesozoicos; Martín-González & Heredia, 2011). La litología es diversa, pero está dominada principalmente por las formaciones kársticas de calizas del Carbonífero y por dolomitas y conglomerados

(IGME, 1994). De acuerdo a la clasificación "USDA Soil Taxonomy", en la cuenca del río Deva-Cares hay cuatro tipos de órdenes de suelos (alfisoles, entisoles, inceptisoles y molisoles), estando la mayor parte del área ocupada por entisoles (IGME, 2005).

2.3 Hidrogeología y geomorfología

La hidrogeología y geomorfología de la cuenca está altamente influenciada por la presencia de macizo kárstico de los Picos de Europa, el cual actúa como una división natural entre el río Deva y su principal tributario, el río Cares. Los principales tributarios de estos ejes son los ríos Quiviesa, Bullón, Urdón, Cares, Casaño, Bulnes y Duje (Figura 2). La altitud y pendiente media de la cuenca son 1100 m y 50.36% (GESHA, 2005) respectivamente, dónde el paisaje es diverso debido a la variabilidad litológica y a los resultados de los procesos kársticos, fluviales, glaciales, periglaciales, nivales y gravitacionales (Adrados et al., 2012; Jiménez-Sánchez et al., 2014), siendo la karstificación el principal proceso en la cuenca. El macizo kárstico de los Picos de Europa contiene el 13% de las cavidades mundiales con profundidades superiores a 1000 m (Ballesteros et al., 2011) y unas 3648 cavidades documentadas que abarcan 355 km (Ballesteros et al., 2015).

2.4 Clima

El clima es principalmente templado oceánico (Rivas-Martínez et al., 2004), pero las condiciones climáticas son variables estacionalmente y espacialmente debido a la proximidad del mar y a los efectos orográficos. La temperatura media anual son 14ºC, aumentando la temperatura media desde las zonas montañosas a las zonas más bajas de la cuenca (Ninyerola et al., 2005). La precipitación media anual son 1300 mm/año, con máximos en los meses de invierno y mínimos en los meses de verano (Ninyerola et al., 2005). La precipitación en forma de nieve es común durante los meses de invierno, representado casi el 20 % de la precipitación anual (Fernández-Giber et al., 2000).

2.5 Hidrología

La karstificación influencia en gran medida la hidrología. Los cursos permanentes son escasos en las partes altas debido a la infiltración que causa una red de acuíferos que emergen a los ríos principales a través de numerosos manantiales. Por su parte, los

cursos temporales en estas partes altas solamente presentan flujo de agua durante eventos de tormenta o en época de deshielo. Los ejes principales de los ríos Deva y Cares están caracterizados por un régimen pluvionival, dónde los caudales más altos se registran al final de otoño y en primavera coincidiendo con la época de deshielo y los caudales más bajos se registran en verano. Los caudales medios de los ríos Deva y Cares antes de su confluencia son 18.5 m^3/s y 22.5 m^3/s respectivamente (Consorcio para el Desarrollo del Oriente de Asturias, 2005).

Los usos del agua en la cuenca han estado estrechamente relacionados con las explotaciones hidroeléctricas desde principios del siglo XX. Además, existen numerosos azudes asociados con molinos y usos de regadío que actualmente tienen caducadas las concesiones de uso. Estas barreras antrópicas longitudinales, junto con las barreras naturales longitudinales presentes en la cuenca, constituyen la pérdida de conectividad de la red fluvial. Otras presiones antrópicas en la cuenca están casi ausentes.

2.6 Socio-economía y ocupación del suelo

La densidad media de población es de 12.33 habitantes/km^2 (Consorcio para el Desarrollo del Oriente de Asturias, 2005). La distribución de la población activa por sectores económicos está caracterizada por un predominio del sector terciario, seguido del secundario y primario respectivamente (GESHA, 2006).

El tipo de vegetación que cubre la mayor parte del área de la cuenca son bosques de frondosas, brezales y matorrales. El bosque autóctono predomina especialmente en la parte sur de del eje del río Deva. Las áreas de suelo sin vegetación se corresponden con el karst de las zonas altas de montaña del macizo central de los Picos de Europa. La agricultura y el pasto se sitúan principalmente cerca de los ejes fluviales. Las áreas urbanas son de pequeño tamaño y los mayores núcleos de población están en las zonas medias de la cuenca y cerca de la desembocadura.

Una gran parte de la cuenca se encuentra situada dentro del Parque Nacional de los Picos de Europa, pero además tienen otras figuras de protección importantes como son cinco Zonas de Especial Conservación (ZECs) de la Red Natura 2000 (European Commission, 1992).

2.7 Flora y fauna

2.7.1 Flora

2.7.1.1 Terrestre

Las especies más frecuentes son características de un clima templado. El bosque por debajo de 400 m de altitud es dominado por *Fraxinus excelsior* L., *Tilia* sp. L., *Corylus avellana* L., *Acer* spp. L., y *Quercus* spp. L., mientras que *Populus* spp. L. *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Fagus sylvatica* L. e *Ilex aquifolium* L. dominan entre los 400 m y los 1100 m. Desde los 1100 m hasta los 1800 m *Betula* sp. L. es la especie dominante, mientras que los prados de montaña y las formaciones rocosas sin vegetación dominan en las zonas altas. La influencia mediterránea produce una comunidad nativa dominada por las encinas *Quercus ilex* L. y *Quercus pyrenaica* Willd.

2.7.1.2 Acuática

Las principales especies del bosque de ribera son *Alnus glutinosa* (L.) Gaertn., *Salíx* spp. L., *Ulmus glabra* Huds., *Fraxinus excelsior* L. y *Corylus avellanea* L. (IH Cantabria-Gobierno de Cantabria, 2011). Las especies de vegetación arbustiva más representativas son *Cornus sanguinea* L., *Euonimus europaeus* L., *Rubus* spp. L, *Tamus communis* L., *Rubia peregrine* L. and *Hedera* spp. L. Las especies herbáceas más frecuentes son *Urtica dioica* L*., Equisetum* spp*.* L*.* and *Polystichum* spp. C. Chr..

La vegetación acuática está principalmente compuesta de briófitos (e.g. *Plagiomnium undulatum* (Hedw.) T.J.Kop and *Rhynchostegium riparioides* (Hedw.) Dixon), hepáticas (e.g. *Conocephalum conicum* (L.) Dum.), macrófitos (e.g. R*anunculus spp* L., *Nasturtium ofcinale* W.T. Aiton and *Apium nodiflorum* (L.) Lag.,) y varias especies de diatomeas (e.g. *Cymbella aff. excisa* Kützing var. *excise*, *Derticula tenuis* Kützing, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Achnanthidium subatomus* (Hustedt) Lange-Bertalot, *Derticula tenuis* Kützing, *Achnanthidium atomoides* Monnier. Lange-Bertalot & Ector y *Achnanthidium pyrenaicum* (Hustedt) Kobayasi).

2.7.2 Fauna

2.7.2.1 Terrestre

Algunas de las especies de mamíferos más características son *Ursus arctos* (Linnaeus 1758), *Canis lupus* (Linnaeus 1758), *Rupicapra rupicapra* (Linnaeus 1758), *Vulpes vulpes* (Linnaeus 1758), *Sus scofra* (Linnaeus 1758), *Capreolus capreolus* (Linnaeus 1758), *Cervus elaphus* (Linnaeus 1758) y *Felix silvestris* (Schreber, 1777). La presencia de varias especies de mustélidos (e.g. *Martes foina*, Erxleben 1777), roedores (e.g. *Eliomys quercinus,* Linnaeus 1766) y murciélagos (e.g. *Myotis blythii*, Tomes, 1857) son comunes.

Las aves son numerosas, entre las que se encuentran *Falco peregrinus* (Tunstall 1711), *Aquila chrysaetos* (Linnaeus 1758), *Prunella collaris* (Scopoli, 1769), *Bubo bubo* (Linnaeus 1758), *Serinus citronella* (Pallas 1764), *Trichodroma muraria* (Linnaeus 1766) y *Cettia cetti* (Temminck 1820) entre muchas otras.

Especies de otros grupos terrestres como invertebrados (e.g. *Lucanus cervus*, Linnaeus 1758) o reptiles (e.g. *Anguis fragilis* Linnaeus 1758) también están presentes.

2.7.2.2 Acuática

Las especies piscícolas que habitan en la cuenca son *Salmo trutta* (Linnaeus, 1758), *Salmo salar* (Linnaeus, 1758), *Anguilla anguilla* (Linnaeus, 1758), *Phoxinus bigerri* (Kottelat, 2007), *Petromizun marinus* (Linnaeus, 1758), *Lampetra planeri* (Bloch, 1784) y en las zonas bajas hay algunas especies de la zona estuarina (e. g. *Platichthys flesus*; Linnaeus, 1758 and Mugilidae).

Los anfíbios característicos del área de estudio son *Bufo bufo* (Linnaeus, 1758), *Pelophylax perezi* (López-Seoane, 1885), *Discoglossus galganoi* (Capula, Nascetti, Lanza, Bullini & Crespo, 1985), *Triturus marmoratus* (Latreille, 1800), *Mesotriton alpestris cyreni* (Wolterstorff, 1932), *Alytes obstetricans obstetricans* (Laurenti, 1768) y *Chioglossa lusitánica* (Bocage, 1864). Las especies de invertebrados son numerosas, destacando la presencia de *Austropotamobius pallipes* (Lereboullet, 1858) con una distribución muy reducida.

Las especies de aves asociadas al ecosistema fluvial son *Riparia riparia* (Linnaeus, 1758), *Alcedo atthis* (Linnaeus, 1758), *Actitis hypoleucos* (Linnaeus, 1758) y *Cinclus cinclus* (Linnaeus, 1758). Otras especies importantes son los mamíferos *Lutra lutra* (Linnaeus, 1758) y *Galemys pyrenaicus* (É. Geoffroy, 1811).

Capítulo III: Mapeo del carácter temporal y permanente de redes fluviales

Este capítulo es una versión editada del artículo publicado en la revista Water Resources Research, 53 (8), 6709-6724, por González-Ferreras, A.M. y Barquín, J. en con el título "Mapping the temporary and perennial character of whole river networks". doi: 10.1002/2017WR020390

Conocer la distribución espacial de los tramos temporales y permanentes a nivel de cuenca es importante para llevar a cabo una gestión integrada de cuenca y una conservación de la biodiversidad de una forma eficaz. Sin embargo, este tipo de información usualmente no está disponible o está incompleta. En este estudio, presentamos una metodología para clasificar todos los segmentos de una red fluvial (cuenca del río Deva-Cares situada en el norte de España) como temporales o permanentes. Esta metodología se basa en una clasificación *a priori* de un subconjunto de segmentos como temporales o permanentes usando datos de campo e imágenes aéreas y posteriormente en la ejecución de modelos Random Forest para predecir la clasificación en el resto de la red fluvial. Las variables independientes y la red fluvial fueron derivadas siguiendo una simulación geoespacial computacional de paisajes fluviales.

Los resultados del modelo mostraron valores altos de precisión, sensibilidad y especificidad en la evaluación del modelo ajustado a los datos de entrenamiento y a los datos del test (≥0.9). Las variables independientes más importantes de acuerdo al índice "Mean Decrease Gini Index" fueron el área de la cuenca, la superficie ocupada por bosques de frondosas, la precipitación mínima mensual en Agosto y la elevación media de la cuenca.

El modelo ajustado predijo 6106 (818 km) segmentos temporales, mientras que 2449 (354 km) segmentos fueron predichos como permanentes. El mapa final de la cuenca del río Deva-Cares en condiciones de caudal bajo (Figura 3c) está compuesto por los segmentos predichos (Figura 3b) y por los segmentos con la clasificación *a priori*

(Figura 3a). El mapa final contiene 7525 segmentos temporales (1012.5 km) y 3731 segmentos permanentes (662.5 km).

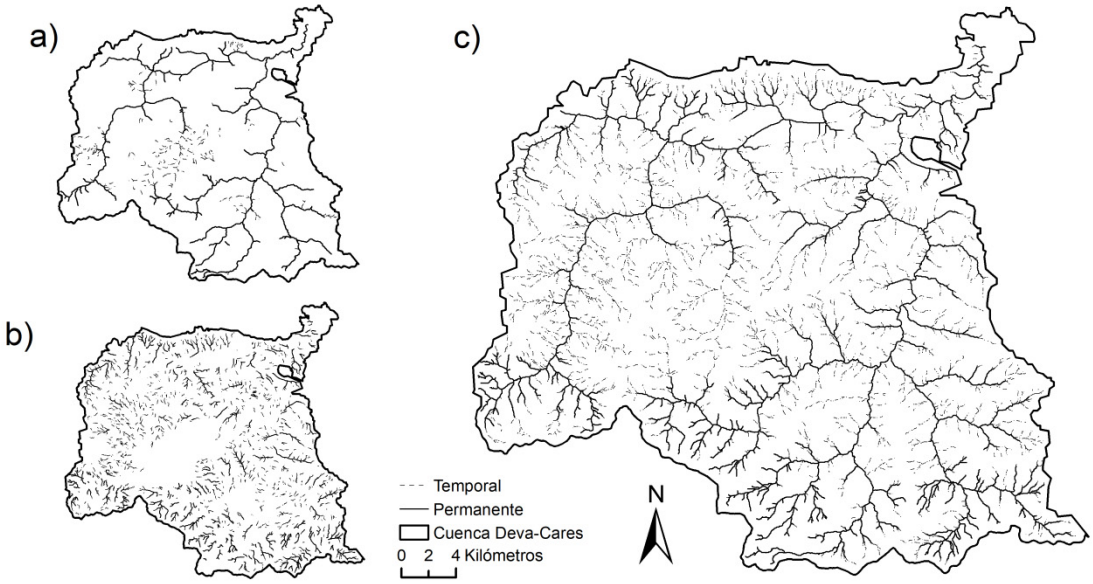

Figura 3. a) Distribución espacial de los segmentos cuya clasificación como temporal o permanente fue asignada mediante datos de campo o imágenes aéreas. b) Distribución espacial de los segmentos cuya clasificación como temporal o permanente fue predicha con el modelo Random Forest. c) Representación de los segmentos temporales y permanentes en toda la red fluvial compuesta de los segmentos predichos (modelados con Random Forest) y los segmentos usados como variables dependientes (asignados mediante datos de campo e imágenes aéreas).

Una validación posterior de los resultados de los mapas usando datos del River Habitat Survey y conocimiento de expertos apoyó la validez de los mapas obtenidos. La metodología propuesta es un método válido para mapear los límites de permanencia del flujo, pudiendo aumentar sustancialmente nuestra comprensión de los límites espaciales entre las interfaces terrestres y acuáticas, mejorando la investigación, gestión y conservación de la biodiversidad y funcionamiento de los ecosistemas fluviales.

Capítulo IV. *Variabilidad espacial de Salmo trutta a escala de red fluvial. ¿Qué variables están influenciando la distribución espacial de la densidad de la población?*

Este estudio, realizado por González-Ferreras, A.M, Alonso, C. y Barquín, J., ha dado a lugar a un manuscrito que se actualmente está en fase de preparación para ser enviado a una revista SCI.

Identificar los patrones de la variabilidad espacial en poblaciones biológicas y los factores que determinan estos patrones a nivel de red fluvial es fundamental para incrementar nuestro conocimiento acerca de los patrones y procesos fluviales. La dispersión de las especies piscícolas está restringida a la red fluvial y, por lo tanto, las características del hábitat acuático y la conectividad longitudinal aparecen como factores clave que pueden influir en los patrones espaciales de la distribución de las especies piscícolas. Sin embargo, la información de estas características clave a la escala de red fluvial usualmente no está disponible o la escala espacial y el tipo de variables ambientales es limitada. En este estudio, se explora el rol que tienen las variables de nicho a diferentes escalas espaciales (cuenca, segmento y tramo) en la determinación de los patrones espaciales de densidad de diferentes clases de edad (alevín, juvenil y adulto) de la trucha común en la red fluvial de la cuenca del río Deva-Cares. Además, también se ha considerado el rol que tienen de la distancia hidrológica y Euclidea y la presencia de barreras infranqueables en los patrones espaciales de densidad de la trucha común. La metodología usada se basa en la selección de variables ambientales con influencia en la densidad piscícola a través de un análisis de correlación de datos y el uso de Modelos Lineales Generalizados (GLM) para analizar la relación de las variables ambientales con la densidad de la trucha común. Por último, se han usado Mantel test y partial Mantel test para evaluar los patrones de la densidad de la trucha común en los puntos de muestreo. El modelo GLM (ver Tabla 1) para alevines (0+) explicó el 25% de la devianza con 3 variables significativas ($p < 0.05$), dónde cada una de las variables se corresponde con cada una de las tres escalas espaciales: densidad de adultos de trucha común (2+), el área total de la cuenca (AREA) y la temperatura media anual en la subcuenca (LC_TEM). En el caso de los juveniles

(1+) solamente la anchura del bankfull (BW_MEA) fue significativa, explicando un 30% de la devianza. La devianza explicada por adultos (2+) fue del 24% con una variable de cuenca (área ocupara por suelo desnudo; MN_DEN), una variable de segmento (anchura del canal activo; ACW_M) y dos variables de tramo (área ocupada por bosque de frondosas dentro de un buffer de 200 m a lo largo del tramo; BF_BLF y velocidad media del agua; V_MEA) seleccionadas.

Tabla 1. Resultados de los modelos GLM para los alevines (0+), juveniles (1+) y adultos (2+) incluyendo las variables predictoras, los parámetros estimados, el error estándar, *t-valor*, *p-valor*, D^2 y valor ajustado de D^2. La variable dependiente fue transformada $\log_{10}$ previamente. Resultados en negrita son significativos ($p < 0.05$).

Clase de edad	Covariables	b(SE)	*t-valor*	Pr(>|t|)	D^2	D^2 ajustado
0+	Intercept	-0.243 (0.272)	-0.894	0.377	0.323	0.248
	2+	8.687 (40.57)	2.141	**<0.05**		
	AREA	-0.001 (≈0.000)	-2.067	**<0.05**		
	LC_TEM	0.142 (0.041)	3.496	**<0.05**		
1+	Intercept	0.018 (1.115)	0.015	0.987	0.359	0.301
	BW_MEA	-0.021 (0.009)	-2.489	**<0.05**		
	I_SUS	0.335 (0.227)	1.476	0.149		
2+	Intercept	1.177 (0.258)	4.562	**<0.05**	0.336	0.241
	ACW_M	-0.022 (0.008)	-2.723	**<0.05**		
	MN_DEN	-0.741 (0.390)	-1.898	0.066		
	V_MEA	2.119 (0.705)	3.006	**<0.05**		
	BF_BLF	-0.761 (0.282)	-2.695	**<0.05**		

Simple y partial Mantel test (ver Tabla 2) revelaron una correlación significativa entre la matriz de disimilaridad de densidades de trucha común y la presencia de barreras infranqueables y los datos ambientales para todas las clases de edad, mientras que la distancia hidrológica solo fue significativa para los alevines.

Tabla 2. Simple y partial Mantel test entre la matriz de disimilaridad Zero-adjusted Bray Curtis para cada clases de edad (0+,1+ y 2+), distancia hidrológica (HD), distancia Euclidea (ED), presencia de barreras infranqueables (PERM_I) y matriz de disimilaridad ambiental (ENV). Resultados en negrita son significativos ($p < 0.05$).

Mantel test		Estadístico de Mantel r
0+	HD	**0.075**
	ED	**0.065**
	PERM_I	**0.078**
	ENV	**0.192**
1+	HD	0.010
	ED	0.015
	PERM_I	**0.082**
	ENV	**0.175**
2+	HD	0.008
	ED	0.002
	PERM_I	**0.105**
	ENV	**0.163**

Mantel test		Controlado por	Estadístico de Mantel r
0+	HD	PERM_I	**0.049**
	HD	ENV	0.082
	HD	ED	0.039
	ED	PERM_I	0.042
	ED	ENV	-0.030
	ED	HD	0.015
	PERM_I	HD	**0.054**
	PERM_I	ED	**0.059**
	PERM_I	ENV	0.108
	ENV	HD	**0.156**
	ENV	ED	**0.17**
	ENV	PERM_I	**0.155**
1+	PERM_I	ENV	**0.082**
	ENV	PERM_I	**0.196**
2+	PERM_I	ENV	**0.137**
	ENV	PERM_I	**0.144**

La densidad de la población de trucha común está espacialmente estructurada por edades y tanto el nicho como la dispersión son factores importantes que influencian la variabilidad espacial de la trucha común a escala de red fluvial. La mayor movilidad de las clases adultas incrementa la importancia de la relación dispersión-conectividad, mientras que el nicho es más importante en las clases de edad inferiores con menos movilidad. La combinación de ambos factores permite explicar una gran proporción de los patrones espaciales de densidad de la trucha común.

Capítulo V. Efectos de la alteración de la conectividad de la red fluvial en la distribución de *Salmo trutta*: perspectivas desde un modelo metapoblacional

Este estudio, realizado por González-Ferreras, A.M, Bertuzzo E., Barquín, J, Carraro, L., Alonso, C. y Rinaldo, A., ha dado a lugar a un manuscrito que se ha enviado para su publicación a la revista Freshwater Biology y que actualmente está en fase de revisión.

La conectividad de la red fluvial es una característica clave de los ríos que afecta a los patrones y procesos de los ecosistemas lóticos. Son pocos los estudios que han considerado como los cambios en la conectividad de los tramos fluviales puede afectar a los atributos del ecosistema a la escala de red fluvial. El uso de modelos de dinámica poblacional de especies clave a escala de red fluvial es esencial para explorar como los efectos de la alteración de los patrones naturales de la conectividad fluvial se pueden propagar a través de la red fluvial. En este estudio, se presenta un modelo metapoblacional para estimar la distribución espacial de las densidades medias de la trucha común, especie caracterizada por ser un depredador con un alto valor ecológico, económico y social. El modelo tiene en consideración la presencia de barreras que limitan la conectividad longitudinal tanto en dirección hacia aguas abajo como aguas arriba. El modelo estima la distribución espacial de densidad de tres clases de edad (alevines, juveniles y adultos) en todos los tramos de la red fluvial basándose en la topología y conectividad de la red y en la dinámica poblacional (e.g. tasa de mortalidad de cada clase de edad, reproducción, dispersión de cada clase de edad y patrones migratorios de reproducción). El modelo fue calibrado con datos empíricos de densidad tomados en muestreos de campo y fue utilizado para explorar diferentes escenarios de conectividad fluvial: escenario 1 (situación actual de la red fluvial con la presencia de todas las barreras longitudinales, el cuál sirvió como base para comparar con el resto de escenarios), escenario 2 (explora la condición de una completa conectividad con la eliminación de todas las barreras longitudinales) y escenario 3 (dónde una sola barrera es eliminada). Además se ha evaluado el efecto en el sesgo en la dirección del movimiento de la especie considerando dos casos: con sesgo en la dirección del

movimiento para adultos hacia aguas abajo y para juveniles hacia aguas arriba (w/bias) y sin sesgo en la dirección de movimiento (w/o bias). El 75% de los resultados del modelo se encuentran dentro de los intervalos de confianza del 95% de los datos empíricos (ver Figura 4; 84.6% para alevines, 69.2% para juveniles y 69.2% para adultos).

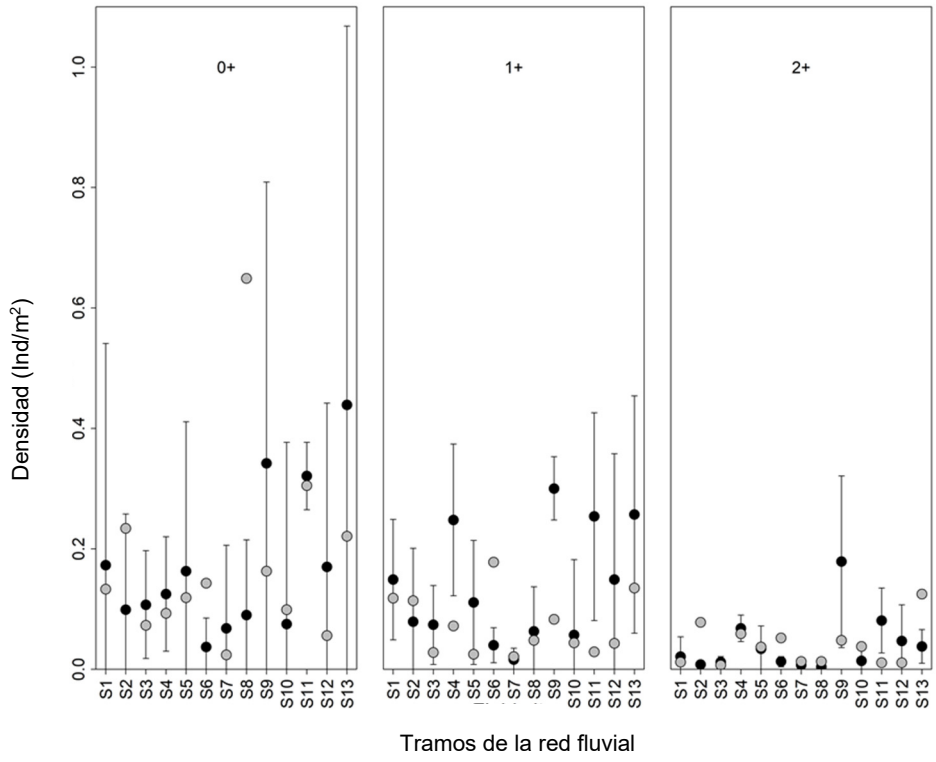

Figura 4. Densidades modeladas (círculos grises) y densidades medias observadas en los datos de campo a través de los años muestreados (círculos negros) para las tres clases de edad de la trucha en los 13 tramos de la red fluvial del río Deva-Cares. Las barras muestran los intervalos de confianza del 95% de las densidades medias observadas basadas en una distribución *t*.

En relación a los resultados de la simulación de escenarios, en el escenario 1 la densidad modelada de alevines es más alta en las zonas de cabecera y la densidad de juveniles y adultos es más alta en los tramos medios (ver Figura 5). La densidad media de la trucha común fue de 0.171 ind/m^2 (0+), 0.064 ind/m^2 (1+) y 0.041 ind/m^2 (2+) considerando el caso del sesgo en el movimiento. El sesgo en la dirección de movimiento tiene un efecto moderado en la densidad piscícola para cada clase de edad, observándose una disminución de menos del 10% en relación a la asunción sin sesgo en el movimiento para este escenario.

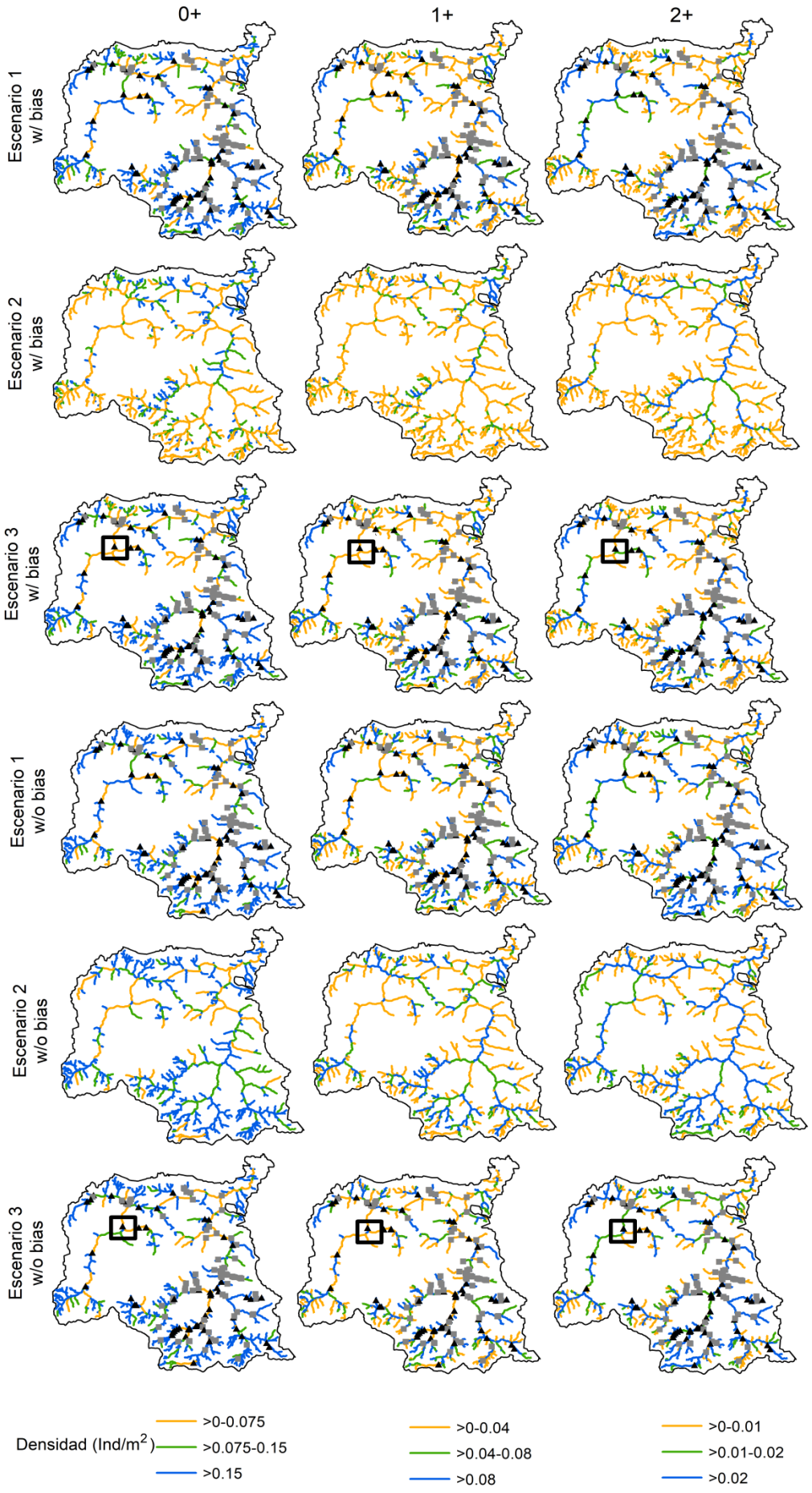
0+
1+
2+
Escenario 1 w/ bias
Escenario 2 w/ bias
Escenario 3 w/ bias
Escenario 1 w/o bias
Escenario 2 w/o bias
Escenario 3 w/o bias
Densidad (Ind/m^2)
>0-0.075
>0.075-0.15
>0.15
>0-0.04
>0.04-0.08
>0.08
>0-0.01
>0.01-0.02
>0.02

Figura 5. Densidad modelada de la trucha común (individuos/m^2) para cada clase de edad, escenarios e inclusión o ausencia de sesgo en el movimiento (w/bias y w/o bias). En el escenario 3, la barrera longitudinal infranqueable eliminada se muestra con un rectángulo negro.

Para el escenario 2 y el caso de sin sesgo en el movimiento, los patrones de densidad fueron similares al escenario 1 sin sesgo en el movimiento (Ver Figura 5 y Tabla 3; las densidades medias fueron de 0.192 ind/m^2 (0+), 0.072 ind/m^2 (1+) y 0.046 ind/m^2 (2+)). Sin embargo, cuando se incluyó el sesgo en el movimiento, la disminución de densidad es relevante en comparación con el caso sin sesgo en el movimiento del escenario 1 (ver Tabla 3). En la ausencia de barreras longitudinales, las densidades modeladas de la clase 0+ son más altas en los tramos de menor área de cuenca, mientras que las densidades de las clases 1+ y 2+ son mayores en las zonas con mayor área de cuenca (ver Figura 5). Aunque esté patrón también se observó en el escenario 1, es más pronunciado en el caso del escenario 2 que contempla una completa conectividad de la red fluvial. En el escenario 2 (w/bias) las densidades medias de la red fluvial son 0.130 ind/m^2 (0+), 0.042 ind/m^2 (1+) y 0.030 ind/m^2 (2+), correspondiéndose con un 24%, 35% y 25% menos en comparación con el escenario 1. En el caso del escenario 2 sin sesgo en el movimiento las densidades medias son un 1% (1+) y 2% (0+ y 2+) que en el caso del escenario 1 (ver Tabla 3).

Tabla 3. Densidades medias de la red fluvial (ind/m^2) para las respectivas combinaciones de escenarios, clases de edad e inclusión o ausencia de sesgo en el movimiento (w/bias y w/o bias). También se representan los valores del escenario 1 para los mismos nodos (aguas arriba/aguas abajo) dónde se produjeron cambios en el escenario 3.

	0+		1+		2+	
	w/ bias	w/o bias	w/ bias	w/o bias	w/ bias	w/o bias
Escenario 1	0.171	0.188	0.064	0.073	0.041	0.045
Escenario 2	0.130	0.192	0.042	0.072	0.030	0.046
Escenario 3 (aguas arriba)	0.010	0.084	0.006	0.034	0.002	0.022
Escenario 1 (aguas arriba)	0.180	0.153	0.075	0.063	0.044	0.037
Escenario 3 (aguas abajo)	0.107	0.158	0.032	0.056	0.025	0.037
Escenario 1 (aguas abajo)	0.102	0.143	0.032	0.052	0.024	0.034

Los resultados del escenario 3 con sesgo en el movimiento (Figura 5 y Tabla 3) mostraron una disminución de la densidad (densidad media: 0.010 ind/m^2 para 0+,

0.006 ind/m^2 para 1+ y 0.002 ind/m^2 para 2+) aguas arriba de la barrera eliminada, mientras que la tendencia opuesta se observó aguas abajo (densidad media: 0.107 ind/m^2 para 0+, 0.032 ind/m^2 para 1+ y 0.025 ind/m^2 para 2+). Estos cambios representan una disminución media del 94% aguas arriba y un incremento del 4% aguas abajo. En general, el escenario 3 produjo una disminución de menos del 5% en la densidad media de la red fluvial. Sin embargo, cabe destacar que los efectos de la eliminación de un solo obstáculo se extienden hasta la localización de las próximas barreras infranqueables tanto aguas arriba como aguas abajo. Cuando no se considera el sesgo en la dirección del movimiento, las densidades medias siguen un patrón similar en relación al escenario 1 para los mismos nodos disminuyendo la densidad aguas arriba e incrementando aguas abajo. En este caso, la disminución aguas arriba es menor (44%) y el incremento aguas abajo es mayor (10%) que en el caso de la consideración de sesgo en el movimiento. El modelo metapoblacional propuesto en este estudio es una herramienta apropiada para evaluar los patrones espaciales de la densidad de la trucha común a escala de red fluvial y para evaluar el impacto de la alteración de la conectividad.

Capítulo VI. *Evidencias genéticas y consecuencias de la conectividad de la red fluvial en una población nativa de Salmo trutta*

Este estudio, realizado por González-Ferreras, A.M, Leal, S, Barquín, J. y Almodóvar, A. ha dado a lugar a un manuscrito que se actualmente está en fase de preparación para ser enviado a una revista SCI.

La conectividad de la red fluvial es una característica clave de los ecosistemas fluviales que puede afectar los patrones y procesos de estos ecosistemas. La alteración de la conectividad en redes fluviales es importante para las dinámicas poblacionales y genéticas de las especies acuáticas. Explorar los efectos de la fragmentación de la red fluvial a través de análisis genéticos es esencial para evaluar el estado de conservación de las especies clave de los ecosistemas fluviales. En este capítulo, hemos investigado las consecuencias genéticas de la alteración de la conectividad en la población nativa de la trucha común en la cuenca del río Deva-Cares. En este estudio se ha investigado i) la variabilidad genética de la trucha común en la cuenca, (ii) la diferenciación genética y la estructura de la población, (iii) las tasas históricas de migración y el tamaño efectivo de la población y (iv) las causas de la diferenciación genética y las características del paisaje fluvial. El ADN fue extraído de las muestras de la aleta adiposa de 197 individuos de 13 localizaciones muestreadas en 2014 distribuidas por toda la red fluvial (Figura 6). 12 loci microsatélites fueron usados en la caracterización de la diversidad genética, de los cuales 2 fueron excluidos por problemas de amplificación y presencia de alelos nulos. Los 10 loci restantes fueron todos polimórficos, con un total de 120 alelos detectados.

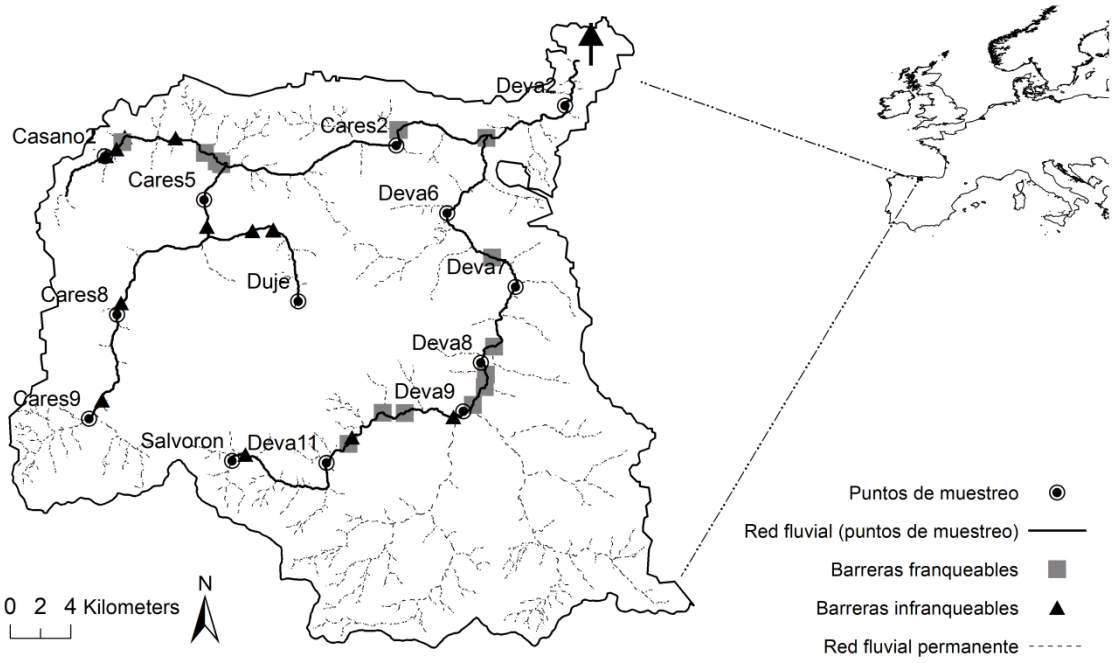

Figura 6. Localización de los puntos de muestreo y barreras longitudinales en la cuenca del río Deva-Cares.

Las localidades de muestreo exhibieron un número medio de alelos comprendido entre 1.900 (Duje) y 6.600 (Deva9), una riqueza alélica media entre 1.859 (Duje) y 6.235 (Deva9) y una heterocigosidad esperada y observada entre 0.224-0.708 y 0.222-0.71, respectivamente. El análisis de la variación genética reveló un gran nivel de diferenciación (F_{ST} = 0.181) con un rango de valores F_{ST} desde 0.002 (Deva8 y Cares2-Deva6) a 0.654 (Salvoron-Duje). Las localidades de Duje, Casano2 y Salvoron son marcadamente diferentes entre sí y tienen un carácter genético distintivo al resto de localidades (F_{ST} = 0.572). En general, las poblaciones aguas abajo de las barreras longitudinales mostraron niveles altos de diversidad genética y valores bajos de F_{ST}, mientras que las poblaciones de cabecera y aguas arriba de las barreras mostraron niveles bajos de diversidad genética y valores altos de F_{ST}.

Se identificaron cinco grupos genéticos y las poblaciones fueron asignadas a cada uno de los grupos: 1) Cares9 and Cares8, 2) Duje, 3) Casano2, 4) Salvoron and 5) Cares5, Cares2, Deva9, Deva8, Deva7, Deva6 and Deva2. Por su parte, Deva 11 se consideró un grupo independiente por su alto contenido de mezcla ente los grupos 4 y 5. Los grupos genéticos identificados se encuentran todos ellos separados por una o más barreras infranqueables (ver Figura 7).

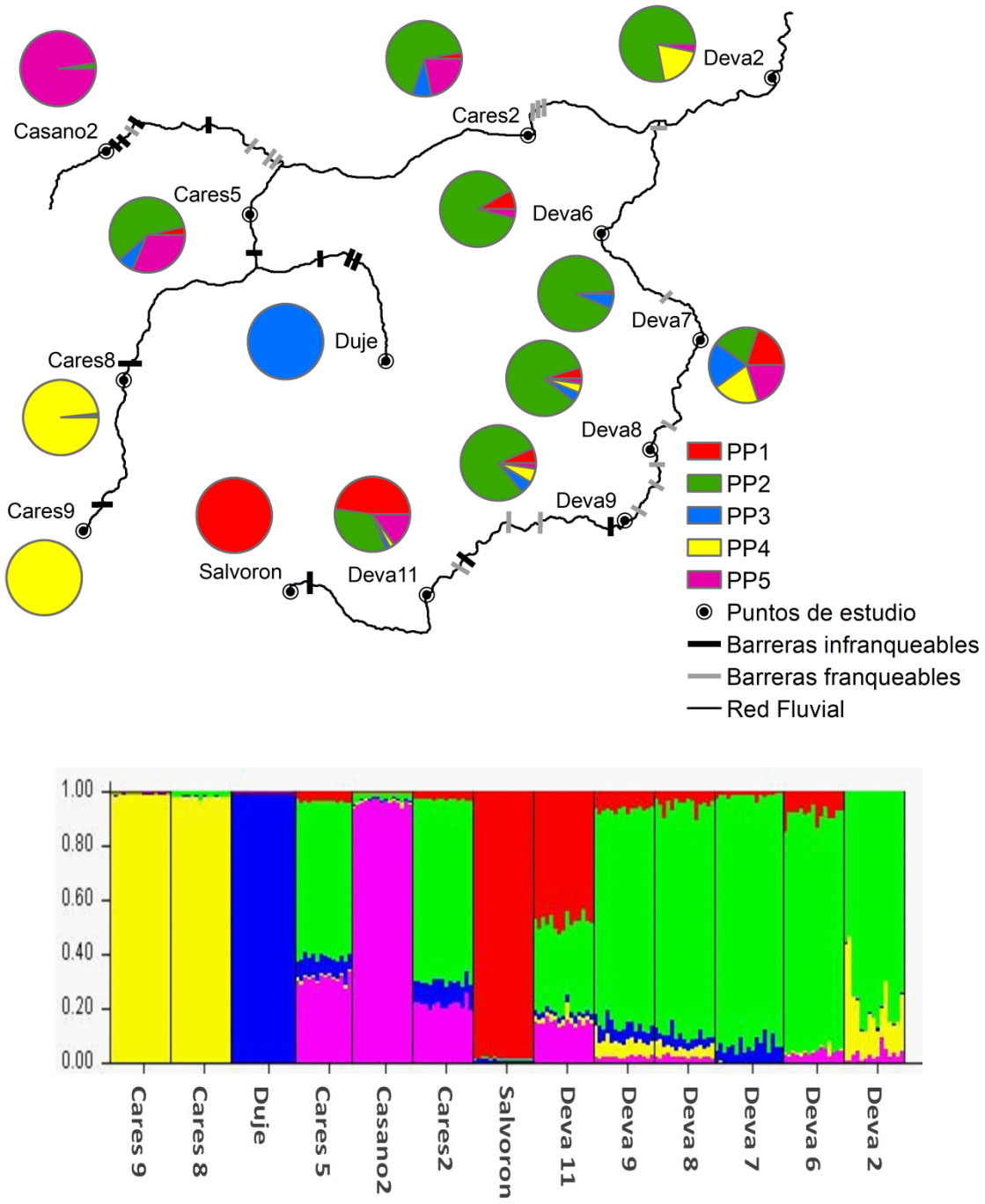

Figura 7. Distribución geográfica de los grupos genéticos identificados por STRUCTURE y los resultados del análisis cluster para K=5. (PP1, PP2, PP3, PP4 y PP5 representan las poblaciones parentales). Las barras de color representan las proporciones de pertenencia de cada individuo a cada grupo.

La obtención de una disminución de la variabilidad genética en las localidades situadas aguas arriba y el flujo de genes sesgado hacia aguas abajo puede ser causa de la fragmentación, ya que los resultados de las migraciones históricas indicaron que el flujo

de genes fue simétrico. La mayoría de las poblaciones mostraron tamaños efectivos de población bajos (solo Cares9, Cares2 and Deva6 mostraron N_e > 50, y solo Cares2 exhibió un N_e > 100), que podrían conducir a una intensa deriva genética y a una alta probabilidad de extinción de las poblaciones. Un cuello de botella histórico fue obtenido para la localización Deva6, mientras que un cuello de botella reciente fue evidenciado para la población Cares8. En cuanto a las causas de diferenciación genética, el aislamiento por barreras infranqueables mostró un rol más importante que la distancia hidrológica en la determinación de la estructura genética de las poblaciones en la cuenca del río Deva-Cares. Los análisis genéticos a escala de red fluvial proveen evidencias del rol de las barreras en la determinación de patrones de diversidad genéticos, destacando la importancia de mantener y restaurar la conectividad longitudinal para conservar las poblaciones de trucha común.

Capítulo VII. Conclusiones generales y futuras líneas de investigación

7.1 Conclusiones generales

Tanto la conectividad espacial como las variables ambientales (i.e. conectividad-dispersión versus nicho) tienen un papel fundamental en la determinación de los patrones espaciales de la trucha común en la cuenca del río Deva-Cares. Los enfoques de análisis y modelado a escala de red fluvial desarrollados en esta Tesis Doctoral proporcionan evidencias importantes sobre cuáles son los principales mecanismos que definen los patrones espaciales de la trucha común en la red fluvial de la cuenca del río Deva-Cares, pudiéndose tomar como un ejemplo importante para otras redes fluviales.

Las poblaciones de trucha en la cuenca del rio Deva-Cares están estructuradas por clases de edad y las diferentes capacidades y preferencias del nicho determinan los patrones espaciales, los cuáles están muy influenciados por la estructura de la red fluvial, la conectividad y la idoneidad de los tramos fluviales por cada diferente clase de edad. Los patrones espaciales de la trucha común (densidades y genética) y los resultados modelados son clave para determinar que la trucha común en la cuenca del río Deva-Cares puede estar funcionando como una metapoblacion. Una serie de razones apoyan este punto de vista de metapoblación: (1) la variabilidad espacial de las variables ambientales a través de la red fluvial genera parches discretos de diferente calidad; (2) las barreras y la conectividad espacial de la red fluvial originan la existencia de parches ocupados y desocupados; (3) las diferentes capacidades de dispersión de las diferentes clases de edad entre los parches de hábitat. La dispersión y la variación el tamaño o calidad de los parches de hábitat evidencian que el tipo de estructura de metapoblación puede ser mainland-island o source-sink. Sin embargo, un significante patrón de aislamiento por distancia también ha sido evidenciado en la población de la trucha común en la cuenca del río Deva-Cares implicando que tanto el modelo de metapoblación como el modelo member-vagrant están presentes en la cuenca y diferentes partes de la red podrían presentar diferentes comportamientos.

Los patrones espaciales y las dinámicas de población de la trucha común en la cuenca del río Deva-Cares están altamente afectados por la fragmentación del hábitat y la pérdida de conectividad debido a la presencia de barreras longitudinales. Esta alteración

modifica sus patrones espaciales naturales y puede influenciar la persistencia de la población de la trucha común en la cuenca del río Deva-Cares evidenciando que las medidas de gestión, planificación y conservación se deben de tomar a la escala de red fluvial.

En los capítulos III, IV, V y VI de la Tesis, hemos investigado la determinación y el modelado de los patrones espaciales de la trucha común en la cuenca del río Deva-Cares a través del rol de la conectividad y el nicho a escala de red fluvial. Las conclusiones obtenidas en esta Tesis Doctoral proporcionan tanto a los científicos como a los gestores una visión importante sobre los patrones espaciales de la trucha común a escala de red fluvial y ayudará en la gestión, planificación y conservación de la especie.

A continuación, se presentan las conclusiones generales para cada uno de estos capítulos de la Tesis Doctoral:

Capítulo III: Mapeo del carácter temporal y permanente de redes fluviales

- El enfoque propuesto para estimar la ocurrencia y extensión de segmentos temporales y permanentes puede ser aplicada en cualquier otra red fluvial considerando la aplicabilidad de nuestras suposiciones. La información del carácter temporal o permanente para la una red fluvial completa usualmente no está disponible, es incompleta o es imprecisa. Por lo tanto, con información relativamente fácil de recopilar y utilizando recursos de datos mínimos (datos de campo, acceso a imágenes aéreas y el enfoque de cuencas virtuales) es posible diseñar un modelo para clasificar los segmentos en temporales o permanentes para redes fluviales enteras.

- La incorporación del conocimiento local y de expertos representa una mejora en el enfoque de mapeo y en los mapas digitales finales.

- El área de cuenca, la superficie ocupada por bosque de frondosas, la precipitación mínima en el mes de agosto y la elevación media de la cuenca son las características de cuenca más importantes que juegan un papel fundamental en la determinación de la distribución espacial del flujo permanente a escala de cuenca.

- La longitud de los segmentos permanentes se considera el hábitat potencial disponible para las poblaciones de trucha común. Sin embargo, la longitud de los segmentos temporales representa un alto porcentaje del total de la longitud de la red fluvial. En la cuenca del río Deva-Cares la mayoría de los tramos temporales se encuentra en las partes alta de la red, y por lo tanto no son tan importantes en el control del a conectividad para las especies piscícolas. Sin embargo, los tramos temporales no deben de ser ignorados en otros estudios a escala de cuenca con el fin de incrementar nuestro conocimiento de la influencia de la variabilidad hidrológica y la intermitencia de la conectividad en comunidades fluviales.

Capítulo IV: Variabilidad espacial de Salmo trutta a escala de red fluvial. ¿Qué variables están influenciando la distribución espacial de la densidad de la población?

- La densidad de la población de la trucha común está estructurada por clases de edad y tanto el nicho como la dispersión son factores importantes que influencian la variabilidad espacial de la densidad de trucha común a escala de red fluvial. La importancia del nicho y la dispersión cambia en función de la clase de edad considerada. La mayor movilidad de las clases adultas incrementa la importancia de la relación dispersión-conectividad, mientras que las características del nicho son más importantes en clases de edad inferiores con menos movilidad.

- La importancia de las variables ambientales a diferentes escalas espaciales difiere entre clases de edad. Las variables ambientales a escala de cuenca, segmento y tramo son más importantes para los alevines y adultos, mostrando una dependencia de los alevines de la densidad de adultos, mientras que los juveniles están más influenciados por las variables de tramo.

- La conectividad principalmente determina la presencia/ausencia de la especie, mientras que las variables ambientales influencias más de capacidad de carga o las densidades medias de la especie en cada tramo fluvial (o parche). Ambos factores deberían de considerarse juntos para entender mejor los patrones espaciales de las densidades de trucha común.

Capítulo V: Efectos de la alteración de la conectividad de la red fluvial en la distribución de Salmo trutta: perspectivas desde un modelo metapoblacional

- El modelo numérico metapoblacional propuesto es una herramienta apropiada para estimar los patrones espaciales de la densidad las diferentes clases de edad de la trucha común en una red fluvial entera y evaluar el impacto de la alteración de la conectividad.

- El modelo metapoblacional basado en topología, conectividad, dinámicas poblacionales y características de dispersión muestra que la heterogeneidad de la densidad de la trucha común es altamente dependiente de la conectividad y de las características de dispersión de la población, así como de la tasa de dispersión de los adultos y la dirección de movimiento.

- La alteración de la conectividad natural de la red fluvial produce cambios importantes en la distribución espacial de la densidad de la trucha común. La eliminación de un solo obstáculo podría tener consecuencias en la densidad de peces incluso en afluentes distantes, mientras que la eliminación de todas las barreras longitudinales en la red fluvial para la migración de los peces restauraría los niveles de densidad de la trucha común a un patrón más natural.

Capítulo VI: Evidencias genéticas y consecuencias de la conectividad de la red fluvial en una población nativa de Salmo trutta

- Las barreras infranqueables tienen un gran efecto en la variación genética de la trucha común que habita en la cuenca del río Deva-Cares actuando como un mecanismo de aislamiento. Las poblaciones situadas aguas abajo de las barreras mostraron altos niveles de diversidad genética y bajos niveles de diferenciación genética, mientras que las poblaciones de cabecera y aguas arriba de las barreras mostraron bajos niveles de diversidad genética y altos niveles de diferenciación genética. El aislamiento por barreras infranqueables mostró tener más importancia que la distancia hidrológica en la determinación de la estructura genética de las poblaciones, aunque un patrón debido a aislamiento por distancia también existe en la cuenca del río Deva-Cares, probablemente causado por las cortas distancias recorridas por los individuos migrantes.

- Una disminución de la variabilidad genética en las localidades aguas arriba y un flujo genético sesgado hacia aguas abajo es posiblemente causado por la fragmentación y el consecuente transporte hacia aguas abajo de algunos individuos por el agua, amplificando la dispersión asimétrica, ya que los resultados de la migración histórica indican que el flujo genético fue simétrico.

- La mayoría de las poblaciones han mostrado un tamaño efectivo de la población pequeño, lo que podría conducir a una intensa deriva genética y una probabilidad de extinción alta.

- La persistencia de las poblaciones depende fundamentalmente de la conectividad y el flujo genético. Se deben de tomar medidas de gestión y conservación para asegurar la viabilidad a largo plazo de las poblaciones de trucha que habitan la cuenca del río Deva-Cares. La población de Deva11 es un área prioritaria para conserva debido a que constituye un importante reservorio genético. Además, las poblaciones de Casano2, Duje y Salvoron deberían de tenerse en plena consideración para su conservación, ya que son susceptibles a bajos niveles de diversidad genética y proveen un flujo genético a otras poblaciones hacia aguas abajo.

7.2 Futuras líneas de investigación

De acuerdo a los objetivos establecidos en esta Tesis Doctoral, hemos identificado importantes cuestiones en la determinación de los patrones espaciales de la trucha común a escala de red fluvial, proporcionando un conocimiento importante y necesario a tener en cuenta en las estrategias de gestión, planificación y conservación de la especie. Además, esta Tesis Doctoral también ha mostrado la existencia de ciertas lagunas del conocimiento y nuevas cuestiones que se deberían de abordar en la investigación futura. A continuación, se describen algunos de los aspectos más relevantes que requieren futuras investigaciones.

- Los resultados obtenidos en esta Tesis Doctoral mostraron una mejora importante de la cartografía digital de tramos temporales y permanentes en la cuenca del río Deva-Cares destacando la necesidad de actualizar la cartografía actual de tramos temporales y permanentes en otras zonas. Se debería de analizar la incorporación de otras variables o métodos para obtener los datos dependientes e independientes para aplicar esta metodología en cuencas dónde dominen otros procesos hidrológicos o nuestras suposiciones iniciales no se cumplan.

- Se deberá de considerar en futuras investigaciones la incorporación de información relativa a la frecuencia y duración del flujo temporal y la presencia de discontinuidades de flujo (e.g. sumideros) para obtener mapas dinámicos que provean más información para delimitar el hábitat disponible para las poblaciones de trucha común.

- Los diferentes resultados obtenidos en el ajuste de los modelos GLM en el capítulo IV considerando las localizaciones donde la especie está presente y ausente o sólo considerando las localizaciones dónde la especie está presente han mostrado la necesidad de llevar a cabo futuros estudios considerando la aplicación de diferentes datos iniciales y evaluar el efecto sobre los resultados. Además, es necesario tener en cuenta las posibles diferencias en los resultados al aplicar nuestra metodología en características diferentes de la población piscícola, como la biomasa en lugar de la densidad. Estos futuros resultados contribuirán al diseño de campañas de campo apropiadas y eficientes para

analizar y modelar datos a escala de red fluvial (e.g. datos balanceados con igual número de presencia y de ausencias).

- Las futuras mejoras del modelo de metapoblación numérico desarrollado en la Tesis Doctoral deberán de tener en cuenta la heterogeneidad espacio-temporal de las variables de la dinámica poblacional. Se deberán de realizar estudios empíricos específicos para obtener datos espacio-temporales de los parámetros del modelo óptimos de acuerdo a las poblaciones locales en el área de estudio. Las variables de dinámica poblacional que necesitan investigarse en especial por la ausencia de datos son aquellas relativas al movimiento y dispersión de la especie (e.g. monitoreo de la distancia y dirección del movimiento para cada clase de edad y proporción de individuos móviles y sedentarios).

- Las fluctuaciones temporales de las variables ambientales también son importantes para determinar cambios en la densidad de peces, las cuáles no se tuvieron en cuenta en este estudio. El modelo numérico metapoblacional se deberá de mejorar incluyendo temporalidad y estocasticidad. Este enfoque futuro permitirá analizar como diferentes situaciones o escenarios no contemplados en esta Tesis Doctoral (e.g. cambio global o alteraciones hidrológicas) pueden afectar a los patrones espacio-temporales de la dinámica poblacional.

- Los estudios genéticos temporales, en adición al enfoque espacial usado en esta Tesis, también deberán realizarse en estudios futuros para identificar un modelo evolutivo en particular para la trucha común en la cuenca del río Deva-Cares.

- Es necesario un análisis de migración actual mediante estudios genéticos para investigar en profundidad el flujo genético en la red fluvial del Deva-Cares. Futuras investigaciones relacionadas con el movimiento de la especie comentadas previamente junto con estos análisis genéticos proporcionarán un conocimiento detallado de la dispersión de la especie a escala de red fluvial. Consideramos que la disponibilidad de esta información es crítica para establecer los límites de las poblaciones compuestas por organismos móviles y para estimar los censos poblacionales.

- Se ha demostrado la existencia de varias poblaciones genéticamente distintas en la cuenca del río Deva-Cares, por lo que estos resultados sugieren que podría

haber más unidades genéticas en la cuenca Deva-Cares debido a la existencia de barreras longitudinales y poblaciones de trucha común en otras partes de la red fluvial que no han sido analizadas. Se deberá de realizar un estudio genético detallado para identificar distintas poblaciones adicionales.

- El conocimiento actual sobre el origen de las poblaciones de trucha común aguas arriba de las barreras naturales en la cuenca del río Deva-Cares es nulo. Se necesitan futuros estudios para identificar si estos tramos han tenido o no previamente trucha común con el objetivo de aplicar estrategias apropiadas de conservación y gestión en estas poblaciones locales y para la metapoblación de la trucha común.

Chapter I

Introduction and background to the research

Chapter I: Introduction and background to the research

1.1 Background and motivation

Freshwaters contain only around 0.01% of the world's water an cover only about 0.8% of the Earth´s surface (Dudgeon et al., 2006). Yet freshwater ecosystems present high species richness in relation to area, supporting almost 6% of all described species (Dudgeon et al., 2006) of which over 12700 species are fish (44% of global fish diversity and 9.1% of global freshwater species; Lévêque et al., 2008; E. Balian et al., 2010). Moreover, freshwaters also provide with multiple ecosystem services basic for the development of human societies (Naiman et al., 2002). However, several anthropogenic impacts of different nature and magnitude are threating both human water security and freshwater biodiversity (Vörösmarty et al., 2010). Threats to freshwater biodiversity can be grouped under five main categories: overexploitation, water pollution, flow modification, destruction or degradation of habitats and invasion by exotic species (Dudgeon et al., 2006). Their combined and interacting influences have caused population declines and extinction risk increases of freshwater biodiversity worldwide (Dudgeon et al., 2006) affecting the composition, structure and function of freshwater communities. Moreover, global change, especially due to changes on temperature and flow, would intensify pressures on freshwater biodiversity (Ruesch et al., 2012).

Freshwater fishes are one of the biological groups more vulnerable to anthropogenic impacts. Global estimates indicate that around 25% of evaluated freshwater fish species are threatened with extinction (i.e. critically endangered, endangered or vulnerable; Vié et al., 2009). These levels are higher in Europe because of a high level of endemism (80% of freshwater fish are endemic), where 37% of evaluated freshwater fish species are threatened with extinction (Freyhof & Wright, 2011). Specifically, the Iberian Peninsula is considered one of the freshwater fish biodiversity hotspots within Europe (Reyjol et al., 2007) characterized by having a large number of endemic species. Nevertheless, they have a low regional diversity compared to other areas in Europe (Carmona et al., 1999), attributed to different historical environmental events, the

location of the Iberian Peninsula and the presence of biogeographical barriers (e.g. the Pyrenees). The Iberian fish fauna is also among the most endangered within the European continent, and fish populations need particular attention for their management and conservation because of their high vulnerability to anthropogenic threats.

The vulnerability of a given freshwater fish population to different threats can be influenced by their biology, dispersal ability, habitat requirements or life-historic characteristics (Angermeier, 1995; Reynolds et al., 2005). Moreover, the vulnerability of riverine biological communities or even whole river ecosystems (including ecosystem functioning) are dependent on the physical environment but also on the biological interdependencies that are established at the community level (e.g., local loss of a predator can induce secondary extinctions; Borrvall & Ebenman, 2006). In this regard, studies focusing on keystone species (Paine, 1966) are important because the variability of the species density has a strong impact on ecosystem processes and biological communities through predation, competition or ecosystem engineering (see Cottee-Jones & Whittaker, 2012 for keystone concepts). Several species linked to freshwater ecosystems have been identified as keystone species in different areas such as *Castor fiber* (Linnaeus, 1758) (Janiszewski et al., 2014), *Chen caerulescens caerulescens* (Linnaeus, 1758) (Kerbes et al., 1990), crayfish (Reynolds, 2011), salamanders (Davic & Hartwell H. Welsh, 2004), anadromous fishes (Willson & Halupka, 1995) and several salmonid fish species (Tzilkowski, 2005). An adequate management and conservation of freshwater ecosystems will need to improve our knowledge on the distribution and on the factors that control the spatial or temporal patterns of these key species.

One of the freshwater fishes considered as a keystone species in previous studies (e.g. Tzilkowski, 2005) and that requires special attention for its ecological and socio-economic importance in the Iberian Peninsula is brown trout (*Salmo trutta* Linnaeus, 1758), species in which this PhD Thesis focuses.

1.2 Brown trout

Brown trout (Figure 1.1) is a salmonid naturally distributed throughout the east Atlantic and north Mediterranean region (Eurasia and North-Africa; Elliott, 1989c see Figure 1.2), although it has been widely introduced by humans to all continents (except the

Antarctic región; B. Jonsson & Jonsson, 2011), being considered nowadays as one of the world´s most invasive fish. The brown trout distribution has been favored by the morphological, physiological and ecological variability of the species (B. Jonsson & Jonsson, 2011). The described taxonomy has been (and still is) controversial due to brown trout phenotypic variability. Some authors (e.g. Kottelat & Freyhof, 2007) described several species, although currently the existence of a single polymorphic species is considered (Elliott, 1989c).

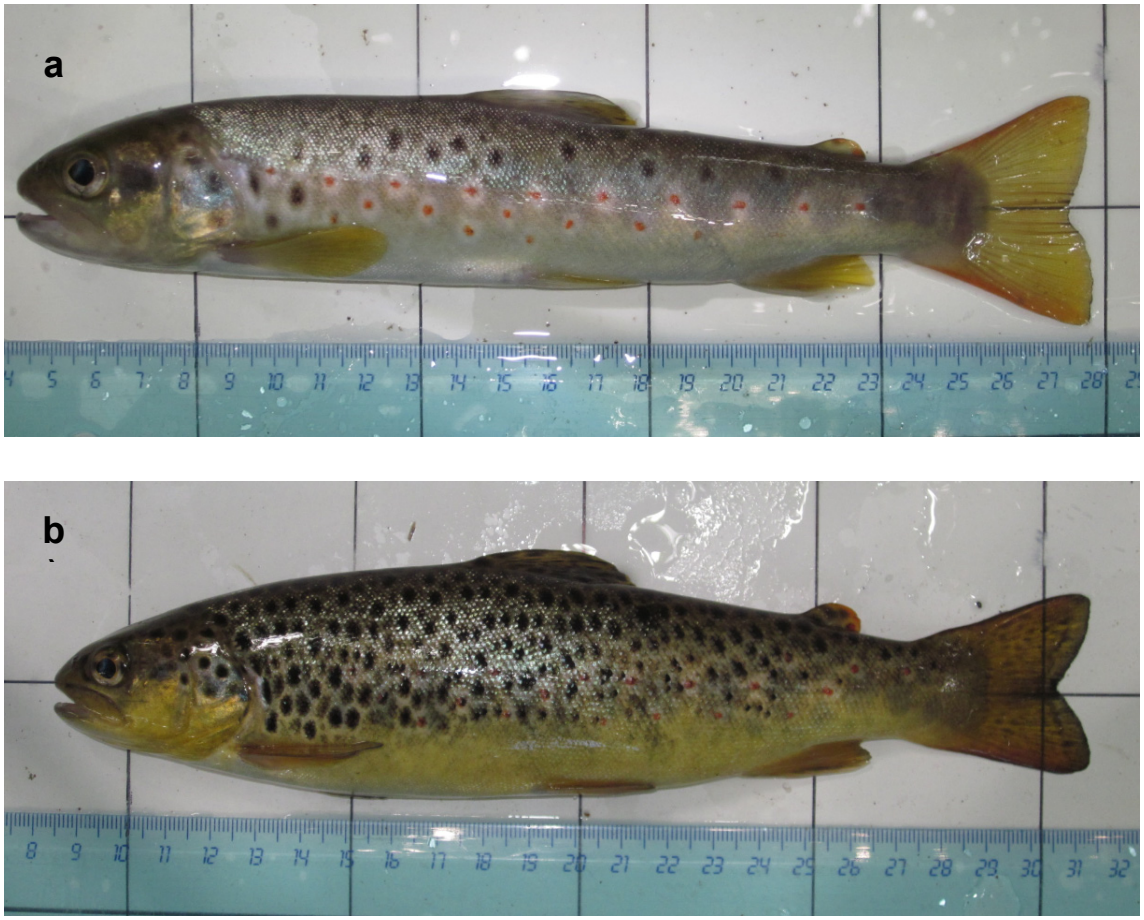

Figure 1.1. Individuals of brown trout in the Deva-Cares catchment: a) Seco river (Deva river tributary) and b) Cares river. Photos taken by the Environmental Hydraulics Institute of the University of Cantabria.

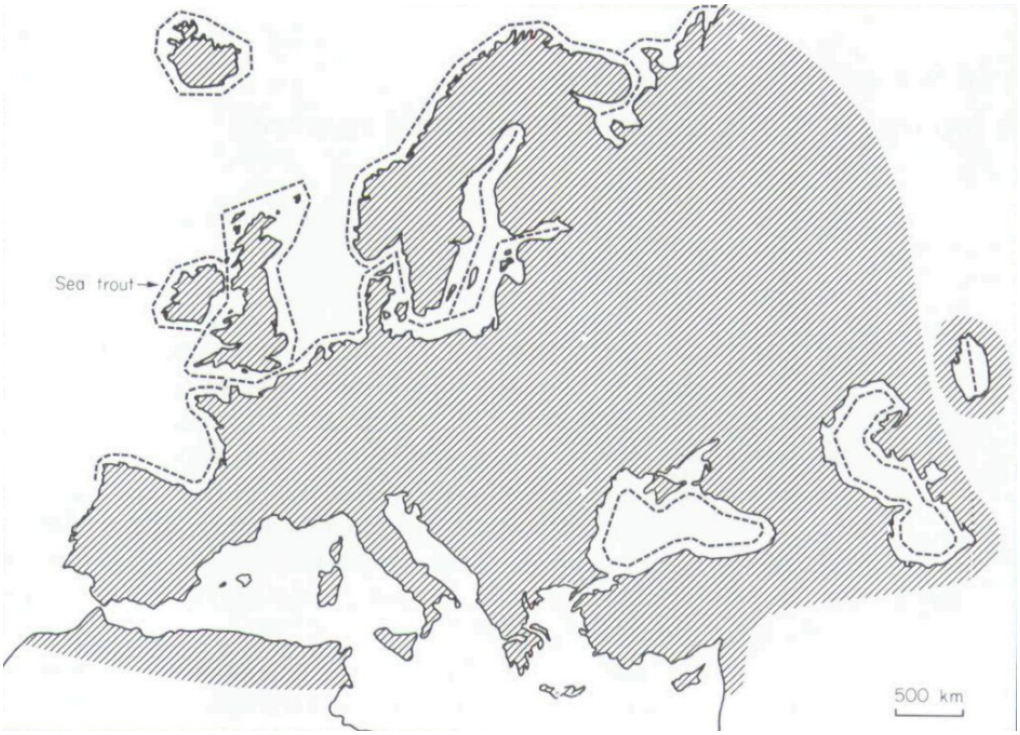

Figure 1.2. Native distribution of brown trout (hatched area). Broken lines indicate anadromous populations. Figure extracted from Elliott (1989c).

Brown trout is a polymorphic species with a high morphological diversity and life history that varies within and among populations (Elliott, 1989b; Milner et al., 2003) with lacustrine, anadromous and resident populations. Phylogenetic analysis by mitochondrial DNA haplotypes have defined 7 lineages: Adriatic, Mediterranean, Marmoratus, Danubian-Black-Sea, Eastern-Danubian, Atlantic, Duero, Tigris and Dades (see Sanz, 2017 and associated references for a review). Geographic distribution and speciation are the results of geological formations and climatic fluctuations.

The Iberian Peninsula represents the southern limit of the species natural distribution and was one of the main glacial refuges during the Quaternary (Hewitt, 1996). Within the Iberian Peninsula, brown trout is naturally distributed in freshwater systems, except some rivers of the Levante and southern Spain and the Guadiana basin (Doadrio, 2002). The Iberian Peninsula brown trout populations are dominated by resident populations. Lacustrine populations are absent in the Iberian Peninsula (Alonso et al., 2012) and anadromous populations (see Figure 1.2) are only present northwards form latitude 42ºN (Hamilton et al., 1989). Mediterranean (ME), Adriatic (AD), Atlantic (AT), and Duero (DU) lineages are present in native Iberian populations (Figure 1.3). The brown trout population of the Deva Cares-catchment is mainly integrated by the Atlantic

lineage and both resident and anadromous populations. However, a study performed by Almodóvar et al. (2008) has also detected haplotypes of the Duero lineage in some rivers of the catchment by fluvial capture phenomena.

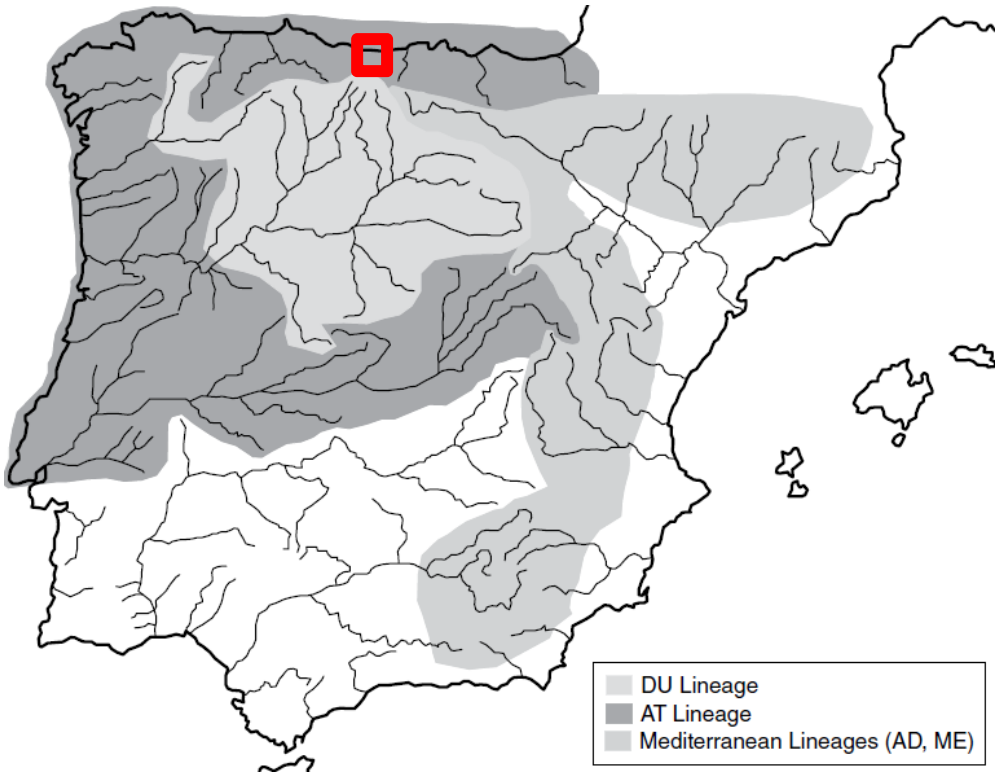

Figure 1.3. Lineage distribution in native Iberian brown trout populations. Figure adapted and extracted from García-Marín et al. (2017) based on Cortey (2005). Red mark represents the approximate location of the Deva-Cares catchment.

Brown trout inhabits mainly clean, cold and well oxygenated waters (Alonso et al., 2012). Spatial and temporal population dynamics are affected by density-dependent (e.g. territorial competition for space and food, disease or parasitism; Milner et al., 2003) and density-independet factors (e.g. climate or hydrology; Alonso et al., 2012), depending on the life stage and environmental conditions (Elliott, 1989b), althought both type of factors might also operate simultaneously. Acoording to the Haldane hypothesis (Haldane, 1956), density-dependent factors would predominate in favorable environments at high densities while density-independent factor would predominate in unfavorable environments at low densities. Previous studies indicate that spatial niche selection by brown trout is size structured (Heggenes et al., 1999; Ayllón et al., 2010) because the different age-classes have different preferences and energy requirements. The most important environmental variables determining habitat suitability for the

species are water depth, velocity, substrate and cover (Heggenes et al., 1999). Brown trout dispersal is restricted to the river network and dispersal patterns of brown trout have been reported to be highly variable composed of both stationary and mobile individuals (e. g. Bridcut & Giller, 1993), being the mobile fraction less abundant (Young et al., 2010). In relation to the different age-classes, dispersal of brown trout fry is limited (Vatland & Caudron, 2015) while adults usually have the highest dispersal rates (Olsson & Greenberg, 2004).

Brown trout has complex, flexible and variable life cycles between populations (Milner et al., 2003). Reproduction takes place from November to January in the Iberian Peninsula (Doadrio, 2002). The age of maturation is variable and could be different between females and males, but the age of maturity at two years is often considered (Alonso et al., 2012). Garcia de Leaniz & Verspoor (1989) observed a mean hybridization rate of 2-3% between brown trout and Atlantic salmon in several watersheds of northern Spain. In that study, the Deva-Cares catchment was also included, but no hybridization was found.

Brown trout spawn in gravels (Haury et al., 1999) and eggs are incubated there for several weeks, hatching later in spring (Klemetsen et al., 2003). A significant relationship between female length and size and number of eggs have been addressed in previous studies (e.g. Nicola & Almodóvar, 2002). Fry stays under the gravel feeding on their yolk sac (Klemetsen et al., 2003) and when most of the yolk is absorbed, fry emerge from the gravel and start feeding on drifting invertebrates (Elliott, 1986) presenting a territorial behavior during this phase (see Figure 1.4 for a simplified representation of the brown trout life cycle). The duration of egg incubation and endogenous larval feeding are water temperature dependent (444 degre day and 220 degre day respectively; Alonso, 2003). The habitat used during the subsequent age-classes vary among populations showing ontogenetic habitat shifts (Klemetsen et al., 2003). Brown trout is an opportunistic feeder and the resources needed by them increase as they grow, being able to exploit gradually larger food items (Klemetsen et al., 2003). The main food source of brown trout are drift and benthic invertebrates and terrestrial preys (P. A. Rincón & Lobón-Cerviá, 1999; Sánchez-Hernández, 2009). Smaller fish can also be predated by older classes (N. Jonsson et al., 1999), and other types of food such as eggs, algae, or substrate remains can also be common on trout diets (Sánchez-Hernández, 2009). In a previous study in an Asturian river in the Cantabrian mountain

range (see Suarez et al., 1988), diptera, epehemeroptera and trichoptera were the most abundant items in the brown trout diet highlighting abundant drift (*Baetis sp*., Leach 1815; Chironomids and simulids), and big benthic prey (*Hydropsyche sp*. Pictet, 1834. and *Rhyacophila sp*. Pictet, 1834).

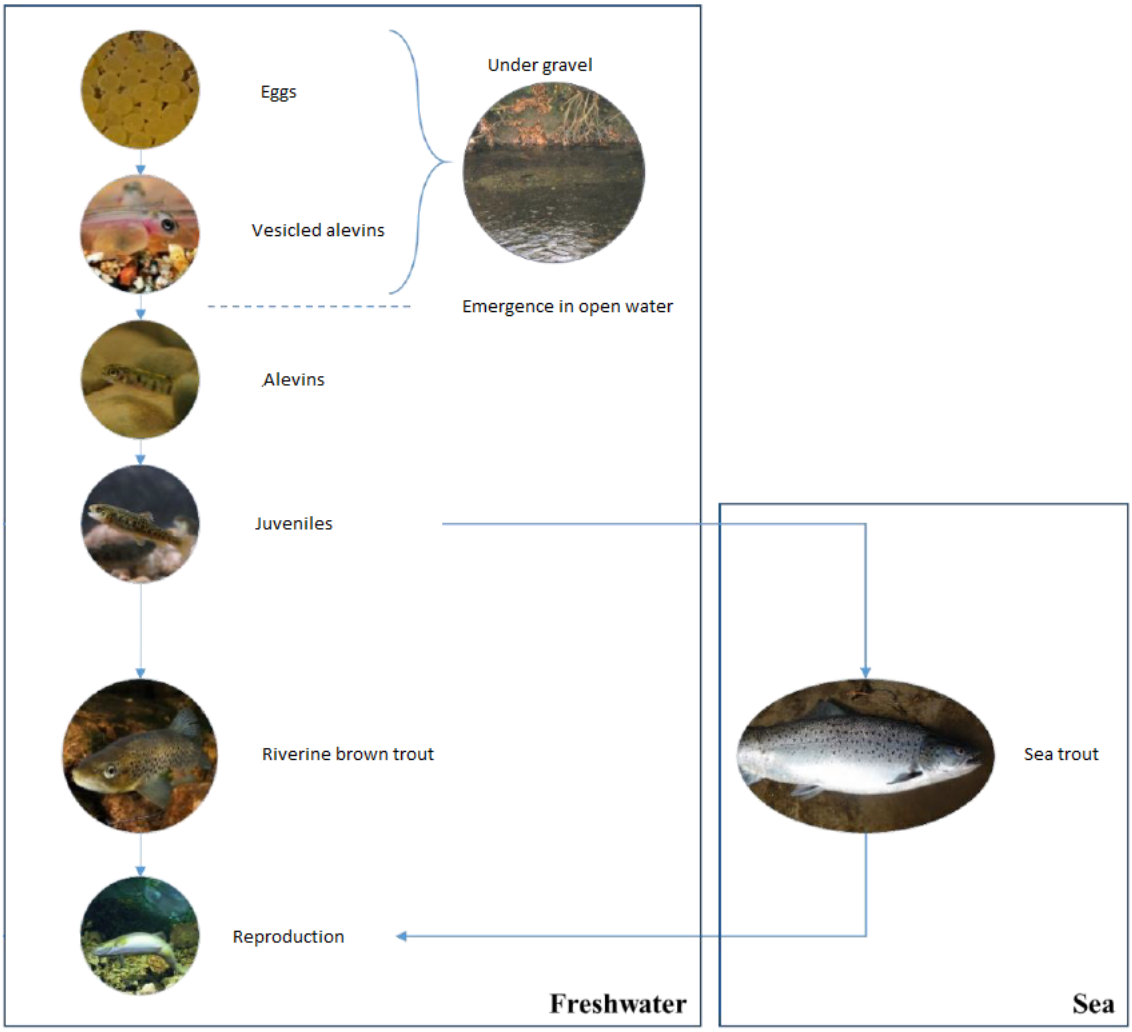

Figure 1.4. Life cycle of brown trout according to life stages and freshwater or sea environment. Figure extracted and adapted from Arévalo (2017).

Brown trout is of great importance from an ecological and socio-economic point of view, as it is the fish top-predator in many freshwater ecosystems (Jensen et al., 2008; Sánchez-Hernández, 2016) and one of the most popular and important sport-fishing species (Almodovar & Nicola, 1998). Despite its importance, Iberian populations have been reduced in recent years and the species is classified as vulnerable in the Atlas and Red Book of the continental fishes of Spain (Doadrio, 2002). The most important threat factors for the species are genetic introgression, overfishing, invasive species, pollution,

global warming, fragmentation and the loss of habitat (Doadrio, 2002; Almodovar et al., 2012; Maceda-Veiga, 2013). Stocking fish from other populations into the Spanish rivers were common in 20th century (Izquierdo et al., 2006) although at the moment most management practices contemplate that specimens should come from the same catchment or contain the greatest possible genetic similarity (Almodovar, 2001). Introgression of foreign genomes into native gene pools have been found in some Spanish Atlantic trout populations, however, not in the Deva-Cares catchment (see Moran et al., 1993; Izquierdo et al., 2006), indicating that the targeted brown trout population in this study has not been genetically disturbed because of past restocking.

In this PhD Thesis, brown trout population will be analyzed from the point of view of niche to river network including dispersal and connectivity, besides considering the importance of potential metapopulation structure for this population.

1.3 The niche concept

The niche is a central concept in ecology and evolution fields. The niche concept was introduced by Grinnell (1917), who defined it as the description of a species habitat requirements. This concept was later redefined by Elton (1927) as the ecological functions of a species. But the formalization of the niche concept was given by Hutchinson (1959, 1978) differentiating two types: (1) fundamental niche which is the n-dimensional hypervolume that occupy a species in the absence of competition and (2) realized niche which is the portion of fundamental niche that occupy a species in presence of competing species. Each dimension represents an environmental variable (biotic or abiotic) important for species persistence. The notion of habitat is derived from the niche concept and can be considered as the geographical realization of the niche (Planque et al., 2011). Moreover, habitat is not constant in time and species can show phenotypically plastic responses (i.e. behavior, life-traits, morphology) to changing environments (Oufiero & Whitlow, 2016).

Species spatial distribution and their abundances are often linked to the breadth and position of their niches (Jorgensen & Fath, 2014). Moreover, size-structured populations can present different niche preferences and niche overlap can occur (Heggenes et al., 1999). Several studies have shown the importance of the niche on freshwater fish populations (Heggenes et al., 1999; Pörtner et al., 2010) and in recent decades, several

statistic model named "Species Distribution Models" (SDM) have been used to associate environmental conditions with species spatial distribution (Leathwick et al., 2005; González-Ferreras et al., 2016). SDMs are defined according to Benito de Pando (2009) as a numerical construction, which defines the ecological relationships between the presence of species and the values of environmental variables influencing their distribution.

Contrary to the niche theory, Hubbell (2001, 2005) proposed the neutral theory assuming that species are functionally equivalent and that their distribution are affected mainly by stochastic processes highlighting the importance of dispersal processes. This theory also have been shown important on freshwater fish studies (Muneepeerakul et al., 2008). The neutral and the niche theories can be considered two extremes of a continuum (Gravel et al., 2006) considering that both approaches have importance for riverine communities although it is often difficult to disentangle the relative influence of each process. Therefore, species spatial patterns could be determined by species niches and dispersal limitation (Jorgensen & Fath, 2014). This concept of dispersion for freshwater populations will be commented in a later section.

Regarding the niche and the species under study in this PhD Thesis, distribution of brown trout populations are influenced by their habitat including both biotic and abiotic factors (Armstrong et al., 2003). On the one hand, several abiotic factors have been shown to influence brown trout populations such as temperature, flow, water depth, substrate, water velocity and cover as the most important, showing also differences between age-classes (see Heggenes et al., 1999; Armstrong et al., 2003 for a detailed description). Habitat suitability curves based on frequency analysis of habitat use by fish have been also used to describe the niche and are often used as the habitat input in habitat-hydraulics model (Heggenes, 1996; Ayllón et al., 2009) using mainly current velocity, substrate and depth as physical variables. Habitat-hydraulic models have been widely used to understand relationship between stream flow and habitat availability for brown trout (and other fish species) and to define the environmental flow (Dunbar et al., 2012), which is really important on alterations of the natural flow regime. On the other hand, abiotic factor more important on brown trout populations are intracohort and intercohort density-dependence (Cattanéo et al., 2002; Parra et al., 2012; Ayllón et al., 2013) and diseases (Schager et al., 2007). It has been widely hypothesized that

environmental drivers operate al larger scale, whereas biotic interactions influence spatial patterns at smaller scales.

1.4 The niche on river networks

Riverine ecosystems possess a four-dimensional nature (longitudinal, lateral, vertical and temporal; Ward, 1989) and are characterized by a dendritic and nested hierarchical structure, connectivity and directionality where river networks are key elements in the landscape integrating hydrologic, geomorphologic and ecologic dynamics (Rodriguez-Iturbe et al., 2009). Within a catchment, rivers usually increase in complexity, flow and size in a downstream direction (Stanford, 2007). The hierarchical spatial structure of riverine ecosystems has long been recognized through river order classifications (Horton, 1945; Strahler, 1964), but a range of different frameworks and classifications have been developed later (see Gurnell et al., 2016 for more information), being the Frissell et al. (1986) hierarchical habitat classification the most used and influential. In this classification, stream systems include all surface waters in a catchment and they are hierarchically organized incorporating, on successively lower levels stream segment, reach, pool/riffle and microhabitat subsystems. This hierarchical structure distinguishes riverine ecosystems from the rest of terrestrial and aquatic ecosystems (Fullerton et al., 2010). The spatial arrangement and the hierarchical organization of habitats influences the distribution and spatial patterns of populations and community interactions (Campbell Grant et al., 2007). For example, diversity is usually higher at confluences an lower reaches (Altermatt, 2013). The river network structure also influences spatial patterns in ecosystem process. For example, autotrophy increases from upstream to downstream (Rodríguez-Castillo et al., 2018). Moreover, different human activities may disrupts these patterns (Branco et al., 2012).

Management and research studies must be conducted at appropriate scales for the question of interest (Fausch et al., 2002). Most of the research and management studies focus on fish species has usually been conducted at small spatial scales (i.e. few hundred meters of river length segments), although in some cases their results may involve erroneous conclusions if they have not considered the scale that match the life history of the species (Fausch et al., 2002). For example, some studies have shown that the habitat used for fish may be much greater than the scale used for management (Cooper & Mangel, 1999) since the spatial arrangement of habitat used for riverine fish

inhabiting river networks for spawning, feeding, rearing or refugee and the connectivity among them affect population vital rates (Schlosser, 1995). Thus, the assessment of single reaches or segments *per se* does not allow understanding the wider effects on a whole river network and these site-based studies may show different results when considering the whole catchment.

The Riverscape paradigm (Fausch et al., 2002), evidenced this mismatch between the scale at which measurements are taken and the biological responses which occurs over whole ecosystems and proposes a continuous view of the river. Riverscape is defined as "a mosaic of freshwater river habitats that is spatially structured and hierarchically organized across multiple scales" (Davis et al., 2018). Previous studies that lead towards this riverscape view were based on the fixed longitudinal zonation of fish species distribution (Hawkes, 1975) which was abandoned by the River Continuum Concept (Vannote et al., 1980) assuming that river networks consist of a continuous gradient of physical conditions from headwaters to a rivers mouth. Posterior theories as the Link Discontinuous Concept (Rice et al., 2001) and the Network Dynamic Hypothesis (Benda et al., 2004) noted the lack of the influence of the tributaries. Poole (2002) proposed the Hierarchical Patch Dynamics perspective highlighting that rivers are formed by patchy discontinuities. Thus, traditional paradigms have been replaced by models and frameworks that take into account the scale, hierarchy, complexity, and heterogeneity of river ecosystems highlighting the importance of network properties such as habitat size or connectivity for an effective and long-term management (Kuemmerlen et al., 2019). There are currently several studies focusing at river network using different modeling techniques such as random forest (Breiman, 2001; Álvarez-Cabria et al., 2017), Spatial Stream Networks (Ver Hoef et al., 2019), patch-based graph (Erős et al., 2012), discrete-continuous hybrid models (Carraro et al., 2018) or ensemble modeling (González-Ferreras et al., 2016) among others. The issues investigated at river network level have been broad: water quality (Álvarez-Cabria et al., 2016; Estévez et al., 2019), hydrological indices (Peñas et al., 2018), hydrological connectivity (Garbin et al., 2019), diversity patterns (Muneepeerakul et al., 2008), bed surface grain size (Snelder et al., 2011), biotic indices (Álvarez-Cabria et al., 2017), ecosystem metabolism (Rodríguez-Castillo et al., 2018), persistence of metapopulations (Mari et al., 2014), climate change an stream temperatures (Isaak et al., 2010) and species spatial distribution (González-Ferreras et al., 2016) among others.

Specifically, the study of González-Ferreras et al. (2016) is considered as a preliminary study that laid the foundation to develop this PhD Thesis (see Appendix: Preliminary study). In this preliminary study, SDMs were used to determine the potential distribution of six freshwater fish species, included brown trout, in several watershed of Northern Spain based exclusively on their niche. Several reasons made this previous study as a key step in the development of this PhD Thesis.

First, only fish data provided by water agencies under nearly natural conditions were used in the SDMs. Evidences found by González-Ferreras et al. (2016) in the design of the training data sets used in the river network models led to the authors to discard the existing data on future studies and forced the collection of new fish data with the proper spatial resolution and design. These authors showed that an unbalanced training dataset, as in the case of brown trout, obtained the lowest predictive regardless model type and assessment methods. This species presented a frequency of occurrence of 94.3% for the study area (100% for the Deva-Cares catchment). Including field sites in the modelling dataset with absences creating a more balanced dataset improved the model performance substantially. These results showed that in order to analyze the population spatial patterns of a generalist species at catchment level, more data should be necessary including information where the species is present, but also absent. To cover the information deficit detected in the existing databases, a specific sampling was designed covering the whole river network (from headwaters to the river mouth) to obtain data representing the spatial variability of the species for this PhD Thesis. Moreover, this previous study was based on presence-absence data of the species, but given the potential importance of brown trout density variability because of their potential impact on ecosystem process and biological communities, we reconsidered it more appropriate to analyze spatial variations in density instead of limiting the analyses to presence-absence distribution.

Second, during the development of this preliminary study, we realized that to determine the actual fish spatial distribution instead of potential distribution, niche characteristics could not be sufficient enough, given the importance of connectivity and dispersal and their influence on the population structure of riverine populations. For this reason, we decided to include these biological processes in subsequent studies to determine the spatial patterns of brown trout at catchment level and analyze which factors were more important.

Finally, with the purpose of analyzing the spatial patterns of the species at a catchment scale in more detail and with the absence of major water quality disturbances, we decided to focus in one of the catchments included in the study of González-Ferreras et al. (2016), the Deva-Cares catchment. This catchment includes several longitudinal barriers both anthropogenic and natural but other major significant pressures are almost absent, making this catchment a suitable area to analyze the role of connectivity and the niche.

1.5 Connectivity and dispersal in river networks

River ecosystems are controlled by hydrological connectivity defined as "the water-mediated transfer of matter, energy, or organism within elements of the hydrological cycle" (Pringle, 2001). Although connectivity comprises interactions along the four dimensions of riverine ecosystems (Ward, 1989) and all of them are important for freshwater species, longitudinal connectivity is the most important for fish regarding the upstream and downstream movements and migrations needed to complete the life cycle between habitat patches (Segurado et al., 2015). Thus, the structure of the river network, the life history and/or the dispersal traits of the species may affect connectivity among aquatic populations (J. M. Hughes et al., 2009). On the one hand, network structure may affect riverine populations by influencing the movement of individuals or transfer of fluxes from upstream to downstream or vice versa (Lowe et al., 2006). On the other hand, life history and dispersal are key elements to understand many population patterns and processes (Lidicker & Stenseth, 1992) including aquatic populations that can disperse strictly along the river watercourses (as brown trout) and populations with overland dispersal in some of their life stage (e.g. some life stages of insects; Chaput-Bardy et al., 2017). For many years, it was assumed that stream dwelling fish as brown trout were sedentary species assuming the restricted movement paradigm of fishes (Gerking, 1959). Now, several studies have invalidated this assumption (Gowan et al., 1994; Rodríguez, 2002) and indicate that trout populations are composed of mobile and stationary individuals, being the latter the predominant proportion (Aparicio et al., 2018).

Four models (Figure 1.5), which are briefly described below, have been used to describe ecological connectivity within and among river networks considering dispersal (for

more information see; J. M. Hughes et al., 2009; Crook et al., 2015; Tonkin et al., 2018):

- **Stream Hierarchy model (Meffe & Vrijenhoek, 1988)**. Connectivity between localities reflects the dendritic structure of the river network assuming minimal out-of network dispersal. Thus, it represents high connectivity within river networks and low connectivity among river networks. It is characteristic of organisms with obligate aquatic dispersal or terrestrial dispersal confined to stream corridors.
- **Death Valley model (Meffe & Vrijenhoek, 1988)**. In this case, communities are composed of stream-dwelling species with no terrestrial dispersion inhabiting isolated patches or of species with high habitat specificity.
- **Headwater model** (Finn et al., 2007). It denotes high dispersal between nearby headwaters streams regardless of hydrological connectivity. It is representative of stream-dwelling species with high habitat specificity and some overland dispersal.
- **Widespread gene flow**: It represents high connectivity among and within networks being characteristic of aquatic species with low habitat specificity and overland dispersal.

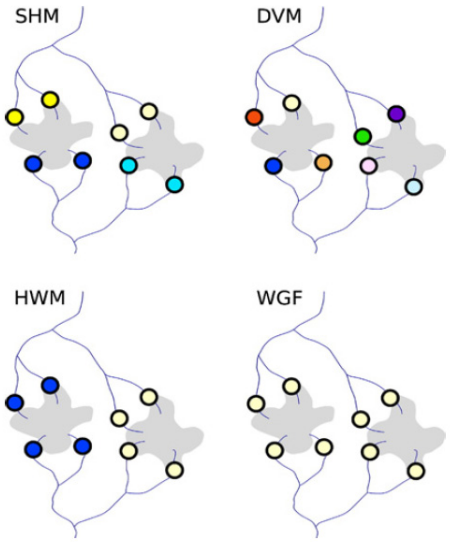

Figure 1.5. Extracted from Crook et al. (2015) representing the four models of ecological connectivity within and among river networks: Stream Hierarchy model (SHM), Death Valley model (DVM), Headwater model (HVM) and Widespread gene flow (WGF). Dots of the same color represent connected populations.

For fish stream-dwelling organisms like brown trout that use river network to their complete life cycle being obligate aquatic dispersal, Stream Hierarchy model is the model more suitable (Tonkin et al., 2018).

Although river networks are naturally fragmented (e.g. waterfalls, rapids), anthropogenic actions have further fragmented these habitats (e.g. dam, weirs). Besides many environmental changes produced by fragmentation on river networks (e.g. alteration of water and sediment fluxes, changes in water temperature; Poff & Hart, 2002), the alteration of longitudinal connectivity affects mainly the quality, quantity and accessibility of habitat patches and dispersal patterns of fish populations (Larinier, 2000) by the barrier effect produced. Hence, fragmentation and loss of habitat may produce several effects in fish populations as extinction of isolated populations (Morita & Yamamoto, 2002), genetic divergence (M. M. Hansen et al., 2014) or asymmetric dispersal (Junker et al., 2012) among others.

Site-specific and local impacts of dams have been extensively investigated (e.g. Lessard & Hayes, 2003; Greathouse et al., 2006; Katano et al., 2006; Gardner et al., 2013). However, these studies might prove insufficient information for understanding consequences at much larger scales (Campbell Grant et al., 2007). Up to date, few empirical studies have addressed how changes on river reach connectivity might affect different ecosystem attributes at a river network scale (but see Ziv et al., 2012; Van Looy et al., 2014). In this regard and despite the influence of habitat and connectivity on fish populations, it is important to incorporate information at catchment scale in studies of spatial patterns of fish populations.

1.6 Metapopulation dynamics

The metapopulation dynamics has been closely developed to the high interest in conservation and management of species mainly in terrestrial ecosystems (Gilpin et al., 1991), but it has been gaining also importance in aquatic ecosystems in recent years (Cooper & Mangel, 1999). The metapopulation term was introduced by Levins (1969) suggesting a population composed of local populations that go extinct and recover, whose dynamics warrant the persistence of the global population. The Levins metapopulation model assumes that all population and habitat patches are identical expressing an unrealistic description of natural metapopulations by their simplicity. Consequently, several modifications to the Levins model have incorporated differences

in local populations and habitat patches (i.e. size and quality; Gilpin et al., 1991). According to Hansky et al. (1997) metapopulation is defined as a "set of local populations within some larger area, where typically migration from one local population to at least some other patches is possible". It should be noted the difference with a single population, defined by Wells and Richmond (1995) as a "group of conspecific individuals that is demographically, genetically or spatially disjoint from other groups of individuals".

Three main conditions define metapopulations: 1) local populations inhabitant discrete habitat patches, 2) the dynamics of occupied patches are not synchronous and 3) there is dispersal events between habitat patches (Rieman & Dunham, 2000; Schtickzelle & Quinn, 2007). Harrison (1991) categorized metapopulation structure in four types: classic, mainland-island and source sink, patchy and non-equilibrium indicating that the magnitude of dispersal between patches and the variance in the quality and size of habitat patches are both elements which determine the type of metapopulation (see Figure 1.6).

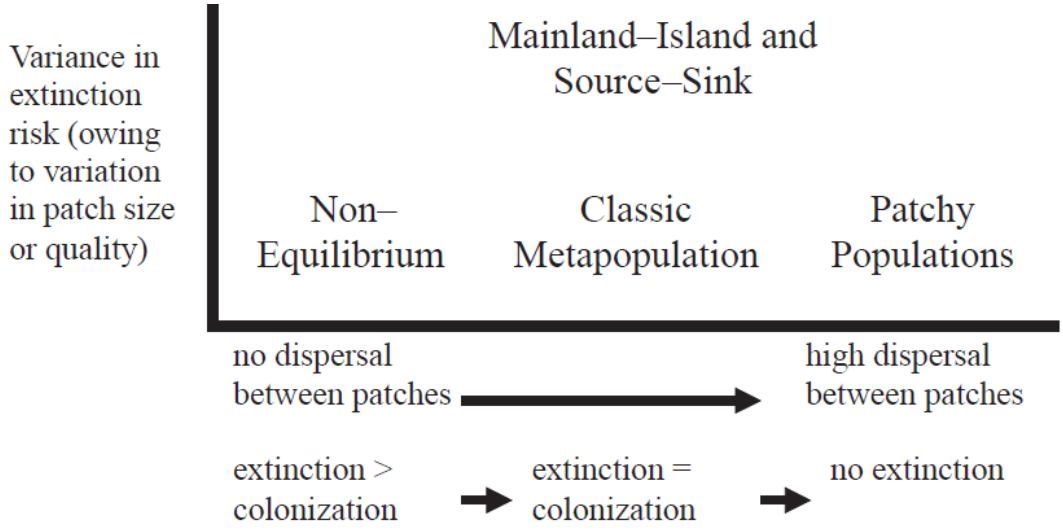

Figure 1.6. Types of metapopulations distinguished by the variation in patch size or quality and the magnitude of dispersal between patches. Extracted from Driscoll (2007).

Below is a brief description of each type of metapopulation:

- **Classic metapopulation.** It is based on the Levins model and considers that all populations are equally likely to go extinct or to be recolonized to ensure persistence.

- **Mainland-island and source-sink metapopulation**. Local extinctions occur in the island or sinks and mainland or sources provide migrants to island and sinks to persist. Mainland-island is characterized by differences in patch size while source-sink is characterized by differences in patch quality.

- **Patchy metapopulation**. It is characterized by high rates of dispersal and recolonization and patches are united into a persistent population.

- **Non-equilibrium metapopulation**. It is characterized by low rates of dispersal and recolonization where subpopulation extinction exceeds the colonization rate.

Fagan (2002) revealed how traditional metapopulation models do not capture the hierarchical dendritic structure of the river and further metapopulation studies (specially fish) have described the spatial effects that river network connectivity exerts on demography and genetics of populations (see e.g. Labonne et al. (2008) and Muneepeerakul et al. (2007) for abstract networks, Fagan (2002) and Muneepeerakul et al. (2008) for real-life settings and Fullerton et al. (2016) for applied/management purposes). Metapopulation theory has been shown relevant for salmonid species, but despite their importance and interest there have been little empirical studies (but see Rieman & Dunham, 2000; Falke & Fausch, 2010). In the specific case of brown trout the studies carried out show diverse results like metapopulation structure (mainland-island or source sink; Østergaard et al., 2003) or isolation by distance (Wright, 1943) showing a continuously distributed population with a decrease in the genetic similarity as the geographic distance increases (Griffiths et al., 2009).

Determine the spatial patterns of brown trout population at catchment scale and analyze the influence of the niche and connectivity are key issues to identify the population structure. Since these populations are threatened by several anthropogenic impacts, mainly by fragmentation and loss of habitat, that can disrupt population structure and influence population persistence, understanding which the main factors are determining the spatial variability of brown trout populations are crucial to accomplish adequate management and conservation actions.

1.7 Objectives of the thesis

The general objective of this PhD Thesis is to determine the spatial patterns of brown trout in the Deva-Cares river network and to analyze the different roles that connectivity and the niche have on determining the spatial variability on this brown trout population. Understanding which the main factors are determining the spatial patterns of this species for the whole river network is an important advancement on river ecology and it is also crucial to preserve and improve the conservation status of the population. Thus, the results obtained from this PhD Thesis will be highly valuable from a scientific point of view and also to design efficient management and conservation strategies at a catchment scale.

The specific objectives of this PhD thesis are focused on the following aspects (Figure. 1.7):

- Determine the available potential habitat for brow trout considering the extent of the perennial network in the Deva-Cares catchment developing a mapping strategy to: (1) estimate the occurrence and extent of perennial and temporary segments in a whole river network at a local catchment scale and (2) determine the main variables which play a fundamental role in determining their spatial distribution (Chapter III).

- Explore and quantify which environmental variables at different spatial scales (catchment, segment and reach) are playing a more important role on determining the density of the brown trout for each age-class and find out whether hydrological and Euclidean distances and presence of impermeable barriers are important on determining brown trout spatial distribution patterns (Chapter IV).

- Develop a metapopulation model to estimate the average spatial patterns of densities of the brown trout for each age-class based on topology, connectivity and population dynamics and explore the effects of connectivity and dispersal on the average spatial patterns of densities of brown trout (Chapter V).

- Investigate the genetic consequences of altered connectivity on the brown trout population in the Deva-Cares river network and describe the patterns of

population structure exploring: (1) the genetic variability of brown trout in the catchment, (2) the genetic differentiation and population structure, (3) the historical migration rates and the effective population size and (4) the causes of genetic differentiation and landscape characteristics (Chapter VI).

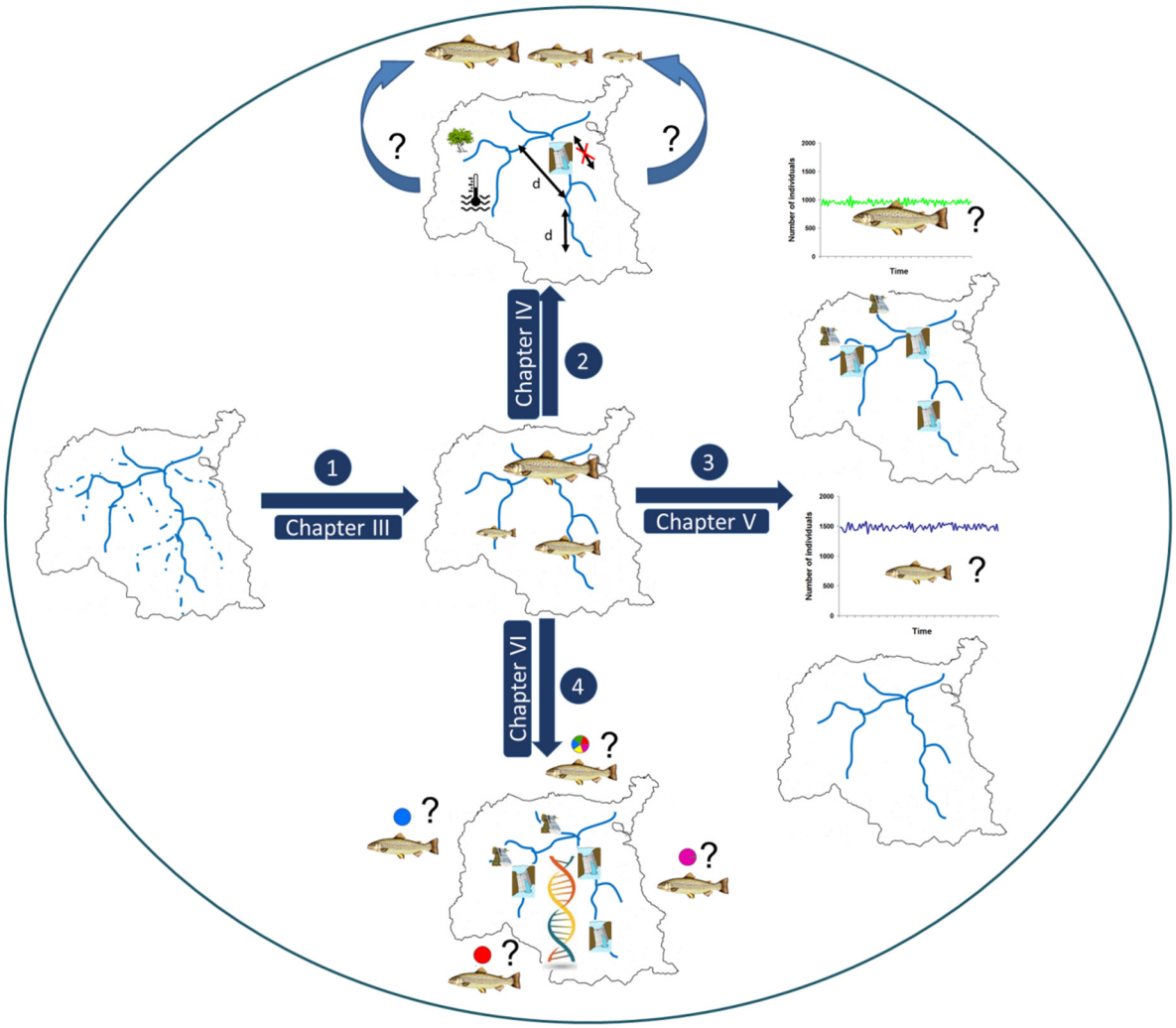

Figure 1.7. Conceptual diagram integrating the different approaches in relation to the specific objectives developed in the different core chapters of the PhD Thesis.

1.8 Layout of the thesis

The structure of the PhD Thesis is organized as follows:

In Chapter I, a general overview and the background to the research objectives are presented first. At the end of this chapter, the general and specific objectives of the PhD Thesis are provided.

In Chapter II, a detailed description of the study area is presented.

The following chapters (III, IV, V and VI) address the objectives of the PhD Thesis. Each chapter includes an abstract, introduction, methods, results, discussion, conclusion and supplementary material section.

A brief synopsis of the investigations conducted in each chapter is described below:

Chapter III. Mapping the temporary and perennial character of whole river networks

In Chapter III, a statistically-based methodology to classify river segments as temporary or perennial is presented for a whole river network (Deva-Cares catchment). This method was based on an *a priori* classification of a subset of river segments as temporary or perennial, using field surveys and aerial images, and then running Random Forest models to predict classification membership for the rest of the river network. The independent variables and the river network were derived following a computer-based geospatial simulation of riverine landscapes. The importance of the independent variables was calculated according to the Mean Decrease Gini Index. A subsequent validation of the mapping results was performed using River Habitat Survey data and expert knowledge.

Chapter IV. Spatial variability of Salmo trutta at a river network scale. What variables are influencing spatial distribution of population density?

In Chapter IV, the role that niche variables at different spatial scales are playing on determining spatial density patterns of brown trout for each age-class (young-of-the-year, juveniles and adults) at a whole river network scale was explored. The role of hydrological and Euclidean distance, and the presence of impermeable barriers on explaining brown trout spatial density patterns was also considered. The methodology used was based on a selection of environmental variables with influence on fish density

data through a correlation analysis and the use of Generalized Linear Models to analyze the relation of the environmental variables with fish density. Mantel test and partial Mantel test were used to look for patterns in the distribution of the densities across field sites.

Chapter V. Effects of altered river network connectivity in the distribution of Salmo trutta: insights from a metapopulation model

In Chapter V, a numerical metapopulation model was developed to estimate the average spatial distribution of the brown trout densities to the whole river network. The model accounts for the presence of barriers that limit longitudinal connectivity in upstream and downstream directions. The model estimates the spatial distribution of densities of three age-classes (young-of-the-year, juveniles and adults) in all river reaches that make up the network based on topology, connectivity and population dynamics (e.g. age-class specific mortality, spawning, age-class dispersal and spawning migration patterns). The model was calibrated against mean observed fish density data from field surveys and was used to detect how modifications to river network connectivity due to removal of longitudinal barriers influenced patterns of brown trout population density.

Chapter VI. Genetic evidences and consequences of river network connectivity on a native Salmo trutta population

In Chapter VI, the genetic consequences of altered connectivity on a native brown trout population in the Deva-Cares catchment was explored. DNA of 197 individuals from 13 locations and 12 microsatellite loci were used to investigate the genetic variability of brown trout in the catchment, the genetic differentiation and population structure, the historical migration, the effective population size and the causes of genetic differentiation and riverscape characteristics.

Finally, general conclusion and future research lines are described in Chapter VII, followed by the Annex and references sections.

Chapter II

Study area

Chapter II: Study area

2.1 Study area

The study area comprises the Deva-Cares catchment, located in Northern Spain and covering an area of 1200 km^2, which drains into the Cantabric Sea (Figure 2.1). Deva-Cares catchment is located primarily in the Eurosiberian biogeographic region, but also spans the Mediterranean region, which is expected to experience significant hydrological impacts because of climate change (e.g. Sánchez de Dios et al., 2009). This catchment is divided into three different administrative regions (Castilla y León (≈150 km^2), Principado de Asturias (≈415 km^2) and Cantabria (≈640 km^2) and a large part of the catchment is located within the Picos de Europa National Park, which is part of the Cordillera Cantabrica mountain range.

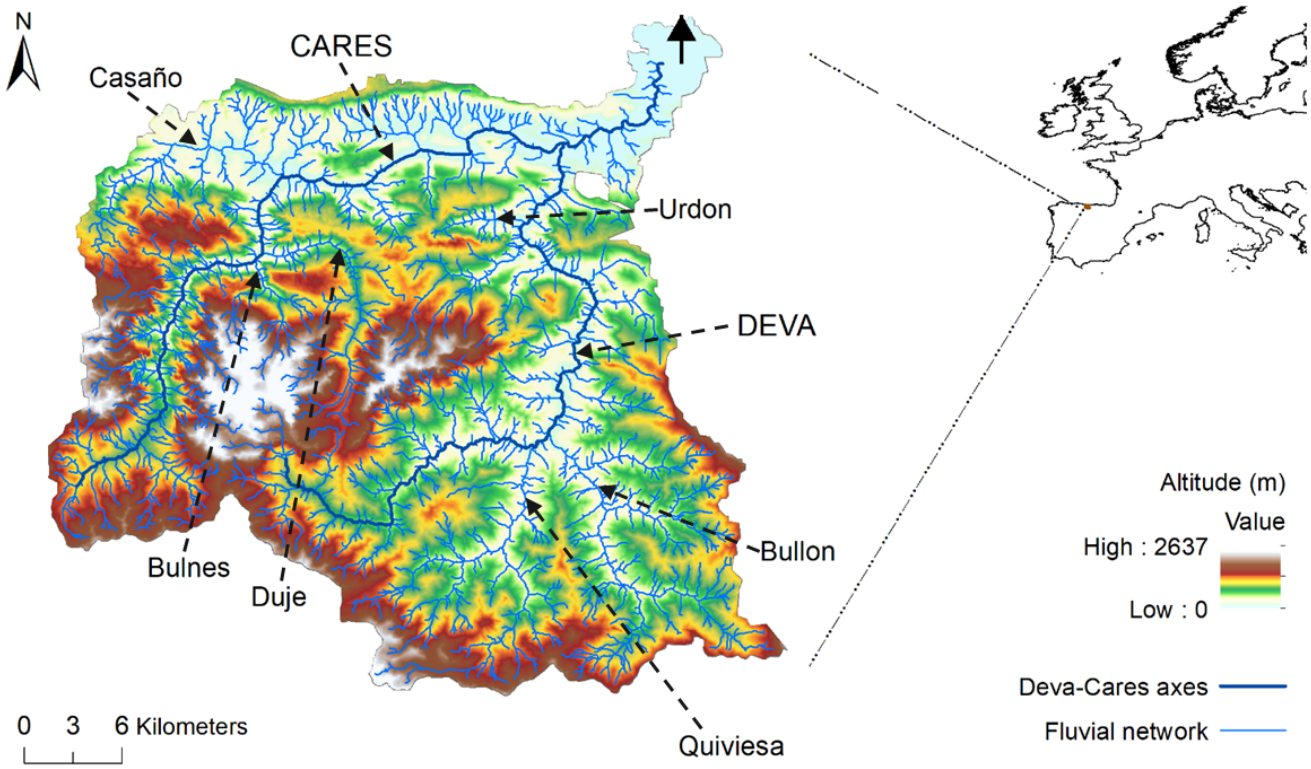

Figure 2.1. Location of the Deva-Cares catchment, main tributaries of Deva and Cares rivers and representation of the digital elevation model.

2.2 Geology, lithology and soils

The study area is located in the Cantabrian Mountains, which has been originated by the successive action of the Hercynian (Carboniferous period) and Alpine orogeny (Terciary period). From a geological point of view, the catchment is located between the Vasco-Cantabrica region (characterized by Mesozoic sediments) and Astur-Galaica region (characterized by the absence or rarity of Mesozoic sediments; Martín-González & Heredia, 2011). Within the Astur-Galaica region the study area is located in the Cantabrian Zone (Julivert & Ribeiro, 1972) comprising the Picos de Europa, Pisuerga-Carrion and Ponga untis. Within the Vasco-Cantabria region, the study area is located on the west part of the Surco Navarro-Cantabro and Norcastellana Platform units. The lithology of the Deva-Cares catchment is diverse (including sandstone, slates and marl) but is mainly dominated by Carboniferous limestone karst formations and by dolomites and conglomerates (see Figure 2.2; IGME, 1994).

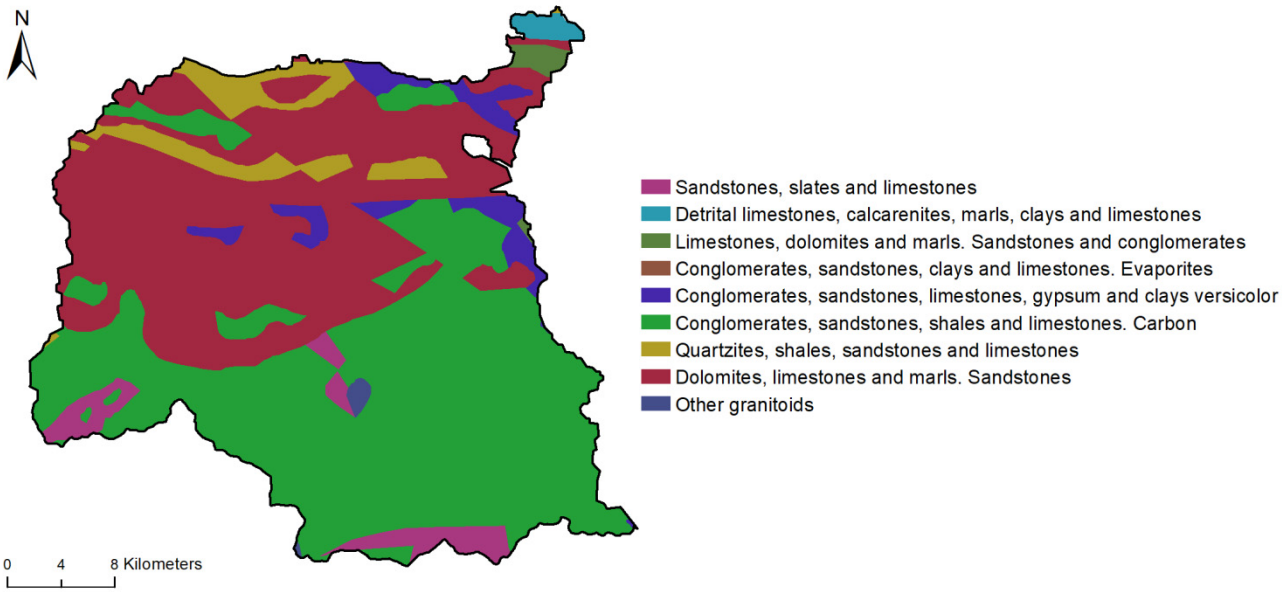

Figure 2.2. Lithological map of the Deva-Cares catchment. Adaptation from IGME (1994).

According to the USDA Soil Taxonomy classification, in the Deva-Cares catchment there are four types of soil orders (alfisols, entisols, inceptisols, and mollisols; see Figure 2.3). Most of the area is occupied by entisols which are characterized by dominance of mineral soils materials and absence of distinct pedogenic horizons (Soil Survey Staff USA, 1999).

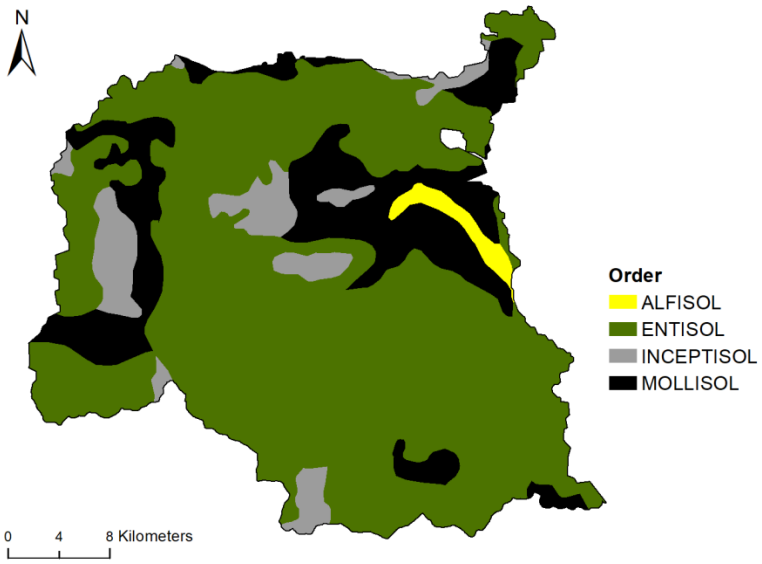

Figure 2.3. Soil orders in the Deva-Cares catchment according to IGME (2005).

2.3 Hydrogeology and geomorphology

The hydrogeology and geomorphology of this catchment is highly influenced by the presence of the karst massif of Picos de Europa, which acts partly as a natural division between the Deva River (length = 64 km) and its main tributary, the Cares River (length = 54 km; see Figure 2.1). Moreover, the massif of Picos de Europa is divided into 3 units by the Cares and Duje rivers (Cares river tributary): Eastern massif, Central massif and Western massif.

From its source in Fuente Dé (1060 m) to the mouth in Tina Mayor estuary, several areas may be differentiated in the Deva-Cares catchment. The main tributaries in this river network are Quiviesa, Bullon, Urdon, Cares, Casaño, Bulnes and Duje rivers (see Figure 2.1). From the source until the town of Lebeña, approximately, the Deva river flows following narrow valleys and high slopes, later the Quiviesa and Bullon tributaries incorporated on the right banks. From Lebeña to Estragueña, the Deva river crosses the Hermida gorge and receives the Urdon river on the left. At the end of the Hermida gorge the valley widens and the Cares river joins to the left margin. The Cares river rises in the Sierra de Cebolleda (1800 m) and crosses glacial valleys until it reaches the Cares gorge from Cain to Poncebos, where Bulnes and Duje (its main tributaries on the right) are incorporated. In Arenas de Cabrales, the Casaño river joins

the Cares and flows parallel to the Sierra del Cuera. The southeastern part of the Deva catchment (drained by Quiviesa and Bullon) and the uppermost part of the Cares tributary have a different hydrological character to the other tributaries within the karst region (Urdon, Casaño, Bulnes and Duje), the former one is dominated by surface runoff flows imposed by old mature forests over shales and conglomerates.

The average altitude of the catchment is 1100 m and the mean slope is 50.36% (GESHA, 2005) where landscape is diverse due to the lithological variability and the results of karstic, fluvial, glacial, periglacial, nival and gravitational process (Adrados et al., 2012; Jiménez-Sánchez et al., 2014; see Figure 2.4). Rivers are characterized by high channel gradients and short channel lengths, with canyons up to 2000 m deep which evidence the significant fluvial process in the catchment (Ballesteros et al., 2011). The Cares and Deva rivers flowing from south to north have carved these narrow canyons (Cares and La Hermida gorges), where it is possible to find fluvial deposits hanging several meters above the current channel (see Figure 2.4a and 2.4b). Evidence of past glaciations are recorded in the higher areas of the massif through erosive (e.g. circus, U valleys) and deposit (e.g. morrenic deposits; see Figure 2.4c) forms of glacial modelling (Obermaier, 1914). At this time there are only some remains of glacier ice in the highest parts (i.e. Jou Negro; Gonzalez-Suarez & Alonso, 1998). There are also numerous evidences of past periglacial landforms (e.g. rocky glaciers) and currently periglacial forms are only recorder above 1900 m (e.g. gelifraction scree; see Figure 2.4d). Nival processes (e.g. erosion and deposition by snow avalanches; see Figure 2.4e) are determined by the intensity and duration of the snow cover which may be eight or nine months above 2000 m, six months above 1500 m and some weeks or days in lower altitudes (José González Trueba & Serrano, 2010). Gravitational processes are mainly related to hill processes (e.g. landslides; see Figure 2.4f) by which the materials can reach the low areas of valleys and rivers. Karstification is the main landscape process in areas with presence of limestone and dolomites, both exokarstic (e.g. sinkholes, poljes; see Figure 2.4g) and endokarstic formations (e.g. caves).

Figure 2.4. Landscape forms by: a) an b) fluvial, c) glacial, d) periglacial, e) nival, f) gravitational and g) karstic processes in the Deva-Cares catchment. a) The "Vega de Liordes" polje (1900 m) in the central massif of Picos de Europa; b) fluvial deposits in La Hoz de Cain, Cares river; c) morrenic deposit in Los Puertos de Aliva; d) slope with gelifraction scree in the Cares trail; e) snow avalanche in Fuente Dé; f) landslide in Brez town. Photos obtained from: a), d) and g) Environmental Hydraulics Institute of the University of Cantabria, b) (Martínez Rodríguez, 2016); c and e) (González Trueba, 2007) and f) (Adrados et al., 2012).

The karst massif of Picos de Europa contains 13% of the world's shafts known to be deeper than 1.000 m (Ballesteros et al., 2011) and some 3648 documented cavities encompassing 355 km of conduits (Ballesteros et al., 2015). At altitudes above 1700 m there is practically no vegetation, and evapotranspiration is below 200 mm. These upper karst zones experience very quick infiltration from snow and rainfall, making this zone one of the main karst aquifer recharge areas (Fernández-Giber et al., 2000). Subsequently, very huge vadose zones develop (well over 1000 m in some areas) before reaching the water table which is very close to the fluvial valleys. The main aquifer discharge occurs through the many springs on the valley bottoms of the main tributaries and even through the river beds, although there are also springs with significantly lower flows in the upper parts of the karst discharging small perched aquifers (Fernández-Giber et al., 2000; Adrados et al., 2012).

2.4 Climate

The climate is mainly temperate oceanic (Rivas-Martínez et al., 2004), but the climatic conditions are highly variable seasonally and spatially, mainly driven by two factors: its proximity to the sea (less than 50 km to the coast in a straight line) and orographic effects (high mountain tops, rising up to 2600 meters above sea level). There are also different zones in the interior (i.e. Liébana valley) that have certain Mediterranean climatological characteristics (GESHA, 2006). Average annual temperature is 14ºC with an increase in the average annual temperature from the mountainous areas to the lower areas of the catchment (Ninyerola et al., 2005; see Figure 2.5a). Mean annual precipitation is 1300 mm/year, showing maximum rainfalls in the winter months and minimum in summer months (Ninyerola et al., 2005; see Figure 2.5b). Snow is common during the winter and accounts for nearly 20% of the annual precipitation, which exceeds 2000 mm above 1000 m.a.s.l. (Fernández-Giber et al., 2000). Cares and Deva axes separated by the central massif of Picos de Europa show a marked difference in terms of precipitation (see Figure 2.5b) with higher values in the Cares area.

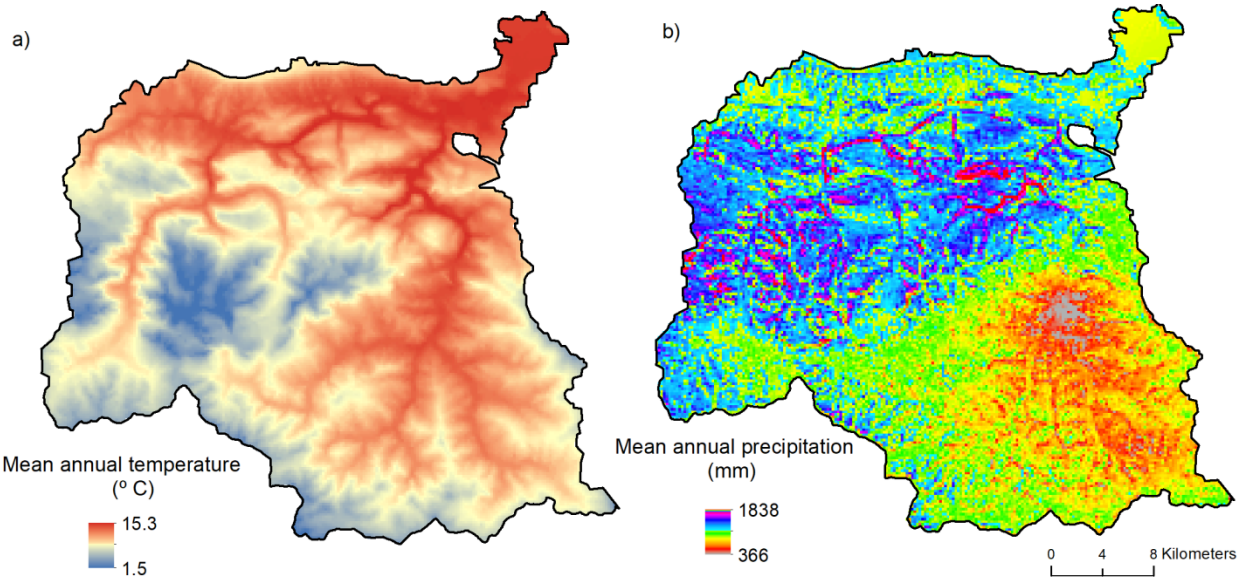

Figure 2.5. Mean annual temperature (a) and precipitation (b) of the Deva-Cares catchment according to Ninyerola et al. (2005).

2.5 Hydrology

Karstification greatly influences hydrology, as already presented in the 2.3 Hydrogeology and geomorphology section. Permanent watercourses are scarce in the highest parts due to the infiltration that causes an immense aquifer networks that emerge to the main rivers through numerous springs. Temporal watercourses in the highest parts present water flow only during intense storm events or the melting snow period. The Deva and Cares axes are characterized by nivopluvial regime. The highest flows are registered at the end of autumn and in spring coinciding with melting snow, while lowest flow is recorded in summer (Consorcio para el Desarrollo del Oriente de Asturias, 2005; MAPAMA, 2015; see Figure 2.6 for more information about monthly flow variability). Rivers in lowest areas are characterized by pluvial regimes. Average annual flow of Deva and Cares river before its confluence are 18.5 m^3/s and 22.5 m^3/s, respectively, (Consorcio para el Desarrollo del Oriente de Asturias, 2005) with minimum and maximum annual flow ranging from 0.06 m^3/s. to 243.89 m^3/s (CHC, 2019). Maximum annual water temperatures are registered in July and August (e.g. Bulnes river 16 ºC) while minimum annual water temperature is recorded during the winter months, (November to April) and usually is less than 8ºC (see http://picoseuropa.ihcantabria.com/ for more information).

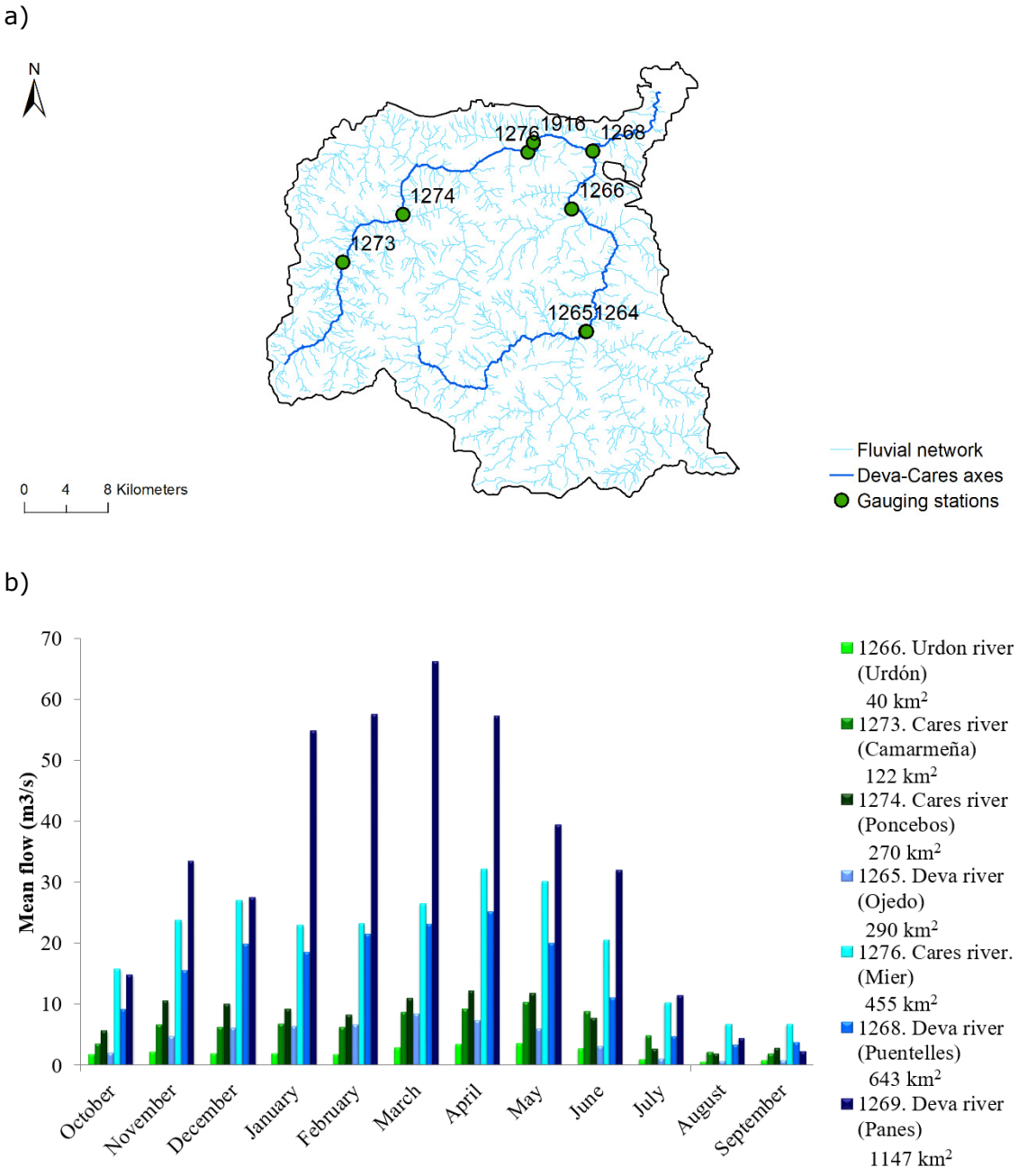

Figure 2.6. a)Map with the location of the gauging stations and b) mean monthly flow recorded in the gauging stations of Deva-Cares catchment (data used from MAPAMA, 2015). The legend of the graphic represents the numeric code of the gauging station, the name of the river, the location and the catchment area. The initial and final year of temporal data series available for each gauging station code are: 1266 (1946-1961), 1273 (1946-1961), 1274 (1994-2014), 1265 (1994-2014), 1276 (1969-2014), 1268 (1970-2014) and 1269 (2012-2013).

Water uses in the Deva-Cares catchment have been closely linked to hydreoelctric explotations since the early 20th century highlighting the Urdon (1912), Canal de Camarmeña from Cain to Poncebos (1921) and Cordiñanes (1995) hidroelectric infraestructures. All of them constitute the largest longitudinal barriers in the catchment and their fish passages have a reduced funcionality. Moreover, there are numerous weirs asociated with old mills and irrigation, many of which have expired water use concessions. Despite the large number of obstacles, the reservoir capacity is low but the loss of longitudinal connectivity produced is high. Aditionally, there are several natural barriers due to the topography that also influence loss of connectivity. Thus, the Deva-Cares river network includes many longitudinal barriers (Figure 2.7) both anthropogenic (102) and natural (37) but other major significant pressures, such as stream habitat alteration or exotic species, are almost absent. This situation makes this catchment a suitable study area to meet the objectives of the PhD Thesis.

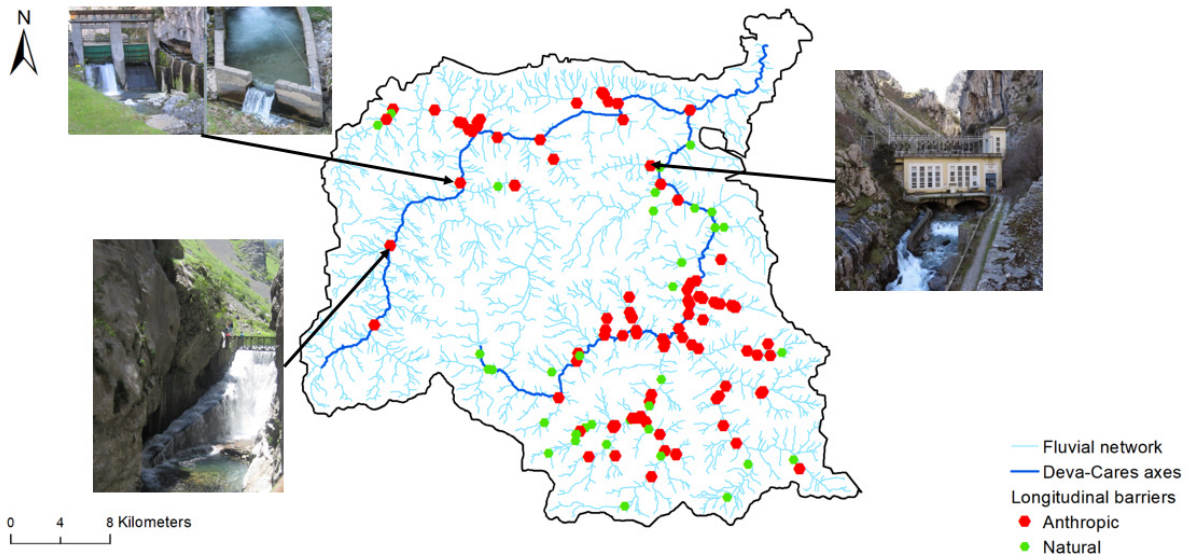

Figure 2.7. Natural and anthropogenic longitudinal barriers in the Deva-Cares catchment.

2.6 Socioeconomics and land cover

The Deva-Cares catchment is under the regional administration of Government of Cantabria, Asturias and Castilla y Leon encompassing 17 municipalities (6 from Asturias, 2 from Castilla y Leon and 9 from Cantabria). Population distribution is conditioned by the relief and the communication routes, representing a mean population in the catchment of 12.33 inhabitants/km^2 (Consorcio para el Desarrollo del Oriente de

Asturias, 2005). The distribution of the active population by economic sectors is characterized by a predominance of the tertiary sector, followed by the secondary and the primary sector respectively (GESHA, 2006). The primary sector is reduced due to the topography and the area that can be dedicated to this sector, while the predominance of the tertiary sector is reflected by tourism (GESHA, 2006), which is increasingly important and highly influenced by the presence of the Picos de Europa National Park (which receive more than 2 million visitors per year; Menéndez de la Hoz, 1999). Some of the most tourist activities of the National Park such as the Cares trail (Figure 2.8), Fuente Dé cable car and funicular of Bulnes are located in the study area. Tourism activities related to the aquatic ecosystem such as fishing and aquatic sports (e.g. canoeing) are also very important.

Figure 2.8. Cares trail between Cain and Poncebos with presence of tourist on the right side. Photo taken by Alexia María González Ferreras.

The vegetation type that covers the largest area in the catchment is broadleaf forest and moors, heathland, scrub and shrubs. Native broadleaf forests are predominant, especially in the southern part of the Deva axes (see Figure 2.9), while other types of forest are hardly represented. Denuded areas are noteworthy, which correspond to the karst on the high mountain areas of the central massif of Picos de Europa. Agricultural and pasture areas are located near the fluvial axes. Urban areas are small and their greatest extensions are found in the middle areas of Cares (e.g. Arenas de Cabrales) and Deva river (e.g. Potes) and near the mouth of the catchment (e.g. Unquera).

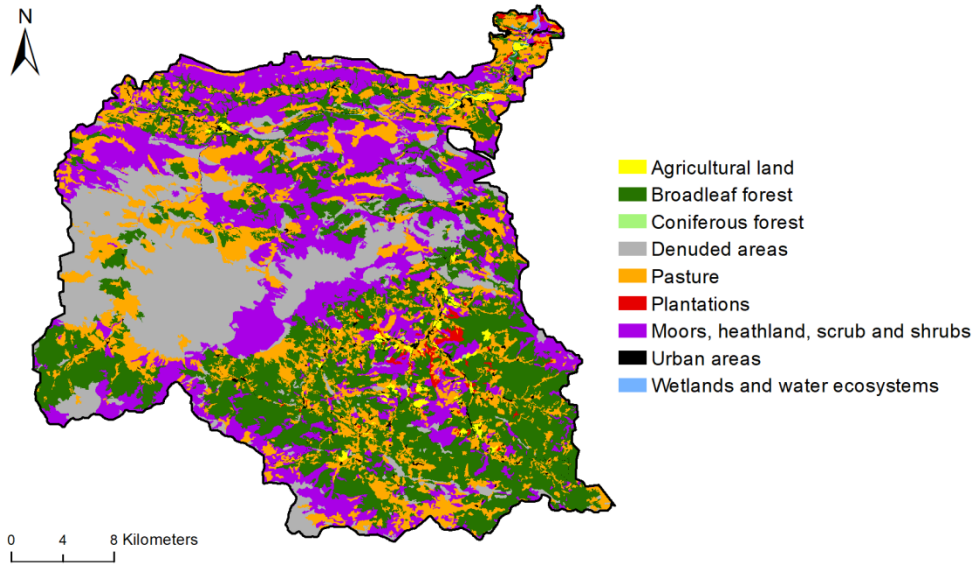

Figure 2.9. Land uses classification in Deva-Cares catchment derived from the Soil Occupancy Information System (SIOSE) developed by the National Geographic Institute of the Spanish Government (IGN, 2011).

In addition to the Picos de Europa National Park, the Deva-Cares catchment has other important protection figures such as five Special Areas of Conservation (SACs) of the Natura 2000 European Network of Nature Protection Areas (European Commission, 1992): Picos de Europa, Picos de Europa Asturias, Liébana, Rio Cares-Deva and Rio Deva (see Figure 2.10).

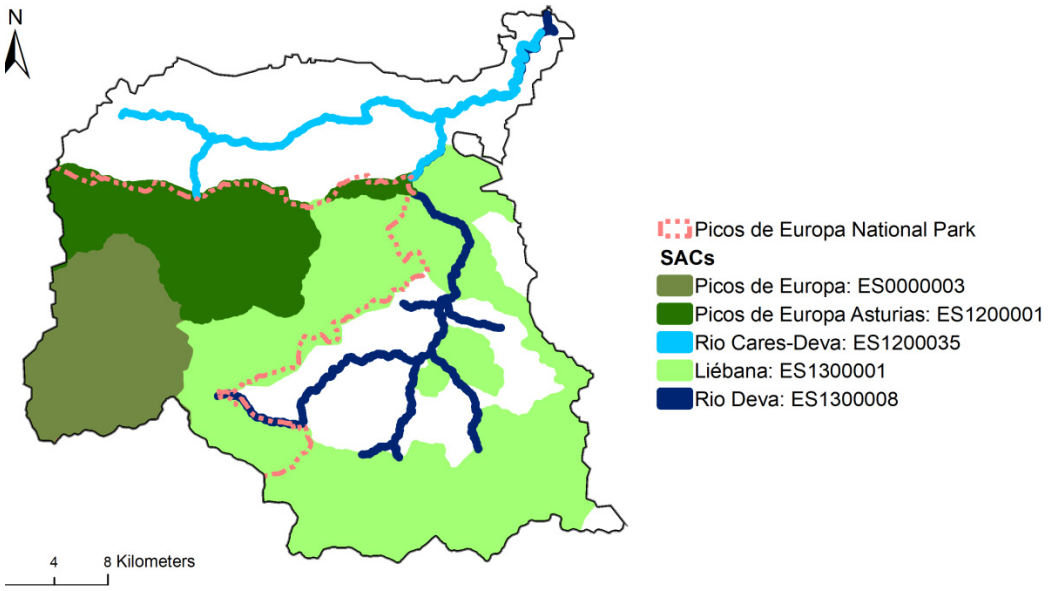

Figure 2.10. Location of the Picos de Europa National Park and SACs (Special Areas of Conservation) of the Natura 2000 European Network of Nature Protection Areas.

2.7 Flora and fauna

The Deva-Cares is located in the south-western limit of the Eurosiberian biogeographic region, constituting the border between Eurosiberian and Mediterranean biota (Rivas-Martínez et al., 2004). This location represents the limit of many populations and communities of flora and fauna (G. Blanco et al., 2005; Jiménez-Alfaro et al., 2012) establishing a biodiversity hotspot.

2.7.1 Flora

2.7.1.1 Terrestrial

The study area is located mainly in the Atlantic European phytogeographic province (Rivas-Martínez et al., 2004). Successions of vegetation vary with altitude generated by climatic conditions, orientation of the slopes and the composition and edaphic structure. The most frequent arboreal species are characteristic of a temperate climate. The natural deciduous forest below 400 m is dominated by *Fraxinus excelsior* L., *Tilia* sp. L., *Corylus avellana* L., *Acer* spp. L., and *Quercus* spp. L., while *Populus* spp. L. *Quercus robur* L., *Quercus petraea* (Matt.) Liebl., *Fagus sylvatica* L. and *Ilex aquifolium* L. dominate between 400 m and 1100 m. From 1100 m to 1800 m *Betula* sp. L. is the

dominant species constituting the last wooded formations, while alpine mountain grasslands and denuded rocks are the dominant features at higher altitudes. The Mediterranean influence produces a native vegetation community dominated by holm *Quercus ilex* L. and *Quercus pyrenaica* Willd.

2.7.1.2 Aquatic

The main wooded species of riparian forests are *Alnus glutinosa* (L.) Gaertn., *Salíx* spp. L., *Ulmus glabra* Huds., *Fraxinus excelsior* L. and *Corylus avellanea* L. (IH Cantabria-Gobierno de Cantabria, 2011). Shrub vegetation is very common whose most representative species are *Cornus sanguinea* L., *Euonimus europaeus* L., *Rubus* spp. L, *Tamus communis* L., *Rubia peregrine* L. and *Hedera* spp. L. The most frequent herbaceous plants are *Urtica dioica* L.*, Equisetum* spp. L. and *Polystichum* spp. C. Chr.. There are several zones where is possible to find some populations of *Woodwardia radicans* (L.) Sm., species included in the Annex II of Habitats Directive (European Commission, 1992). In the lower area of the basin there are some estuarine species due to the influence of the estuary.

Riverbed vegetation is mostly composed of bryophytes (e.g. *Plagiomnium undulatum* (Hedw.) T.J.Kop and *Rhynchostegium riparioides* (Hedw.) Dixon), hepatics (e.g. *Conocephalum conicum* (L.) Dum.), macrophites (e.g. R*anunculus spp* L., *Nasturtium ofcinale* W.T. Aiton and *Apium nodiflorum* (L.) Lag.,) and many diatomaceous species (e.g. *Cymbella aff. excisa* Kützing var. *excise*, *Derticula tenuis* Kützing, *Achnanthidium minutissimum* (Kützing) Czarnecki, *Achnanthidium subatomus* (Hustedt) Lange-Bertalot, *Derticula tenuis* Kützing, *Achnanthidium atomoides* Monnier. Lange-Bertalot & Ector and *Achnanthidium pyrenaicum* (Hustedt) Kobayasi).

2.7.2 Fauna

2.7.2.1 Terrestrial

The great diversity of habitats that exist in the study area results in a high fauna richness. Some of the most characteristics mammals are *Ursus arctos* (Linnaeus 1758), *Canis lupus* (Linnaeus 1758), *Rupicapra rupicapra* (Linnaeus 1758), *Vulpes vulpes* (Linnaeus 1758), *Sus scofra* (Linnaeus 1758), *Capreolus capreolus* (Linnaeus 1758), *Cervus elaphus* (Linnaeus 1758) and *Felix silvestris* (Schreber, 1777). The presence of

several species of mustelids (e.g. *Martes foina*, Erxleben 1777), rodents (e.g. *Eliomys quercinus,* Linnaeus 1766) and bats (e.g. *Myotis blythii*, Tomes, 1857) are common in the study area.

Birds that inhabit the area are numerous and some species that can be found in the area are *Falco peregrinus* (Tunstall 1711), *Aquila chrysaetos* (Linnaeus 1758), *Prunella collaris* (Scopoli, 1769), *Bubo bubo* (Linnaeus 1758), *Serinus citronella* (Pallas 1764), *Trichodroma muraria* (Linnaeus 1766) and *Cettia cetti* (Temminck 1820) among many others.

Numerous species of other terrestrial groups such as invertebrates (e.g. *Lucanus cervus*, Linnaeus 1758) or reptiles (e.g. *Anguis fragilis* Linnaeus 1758) are also present in the area.

2.7.2.2 Aquatic

The Deva-Cares catchment presents numerous species linked to fluvial ecosystems. Fish species inhabiting the catchment are *Salmo trutta* (Linnaeus, 1758), *Salmo salar* (Linnaeus, 1758), *Anguilla anguilla* (Linnaeus, 1758), *Phoxinus bigerri* (Kottelat, 2007), *Petromizun marinus* (Linnaeus, 1758), *Lampetra planeri* (Bloch, 1784) and in the lower part there are some species coming from the estuary (e. g. *Platichthys flesus*; Linnaeus, 1758 and Mugilidae; see Figure 2.11). Freshwater fish species are all of them native and are contemplated in several national and international legislations and regulations incorporating specific measures for their management and conservation (see Table 2.1).

Amphibians characteristic of the study area are *Bufo bufo* (Linnaeus, 1758), *Pelophylax perezi* (López-Seoane, 1885), *Discoglossus galganoi* (Capula, Nascetti, Lanza, Bullini & Crespo, 1985), *Triturus marmoratus* (Latreille, 1800), *Mesotriton alpestris cyreni* (Wolterstorff, 1932), *Alytes obstetricans obstetricans* (Laurenti, 1768) and *Chioglossa lusitánica* (Bocage, 1864). Invertebrate riverine species are numerous, highlighting the presence of *Austropotamobius pallipes* (Lereboullet, 1858) which is located in some streams and tributaries of scarce entity presenting a very restricted distribution.

There are several birds species associated to fluvial ecosystems such as *Riparia riparia* (Linnaeus, 1758), *Alcedo atthis* (Linnaeus, 1758), *Actitis hypoleucos* (Linnaeus, 1758)

and *Cinclus cinclus* (Linnaeus, 1758). Other important species linked to the aquatic environment are the mammals *Lutra lutra* (Linnaeus, 1758) and *Galemys pyrenaicus* (É. Geoffroy, 1811), both included in the Annex II of Habitats Directive (European Commission, 1992).

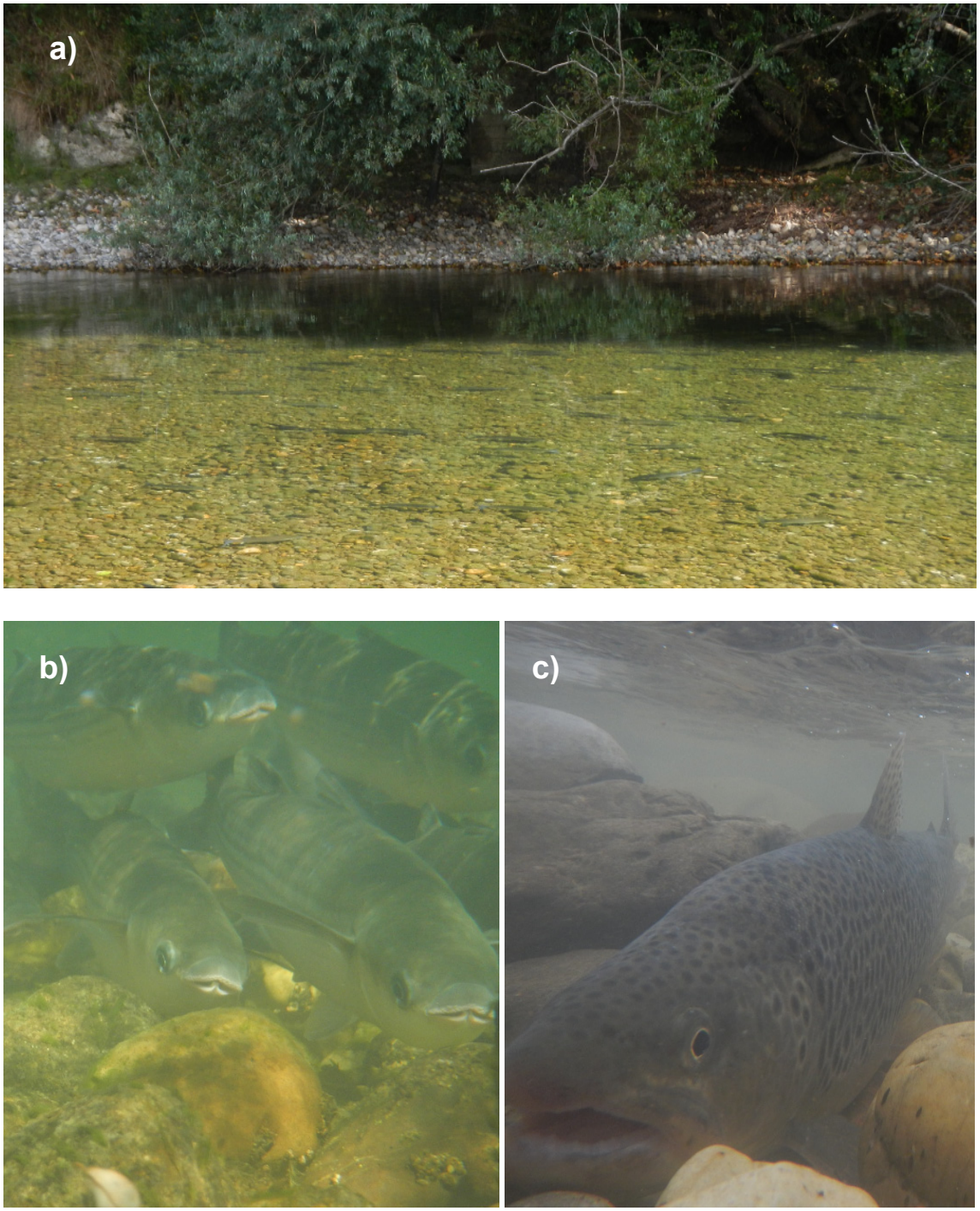

Figure 2.11. Mugilidae in the lower part of the Deva river (a and b). *Salmo trutta* in the Cares river (c). Photos taken by the Environmental Hydraulics Institute of the University of Cantabria.

Table 2.1. National and international legislation and regulations where freshwater fish species of the Deva-Cares catchment are considered.

Species	Normative
Salmo trutta	**National** • 1095/1989 Royal Decree of 8 September, in which the species hunted and fished are declared and requirements established for their protection. • 1118/1989 Royal Decree of 15 September, in which the species hunted and commercially fished are determined and requirements developed with regard to these. • Vulnerable in the Red Book of the Vertebrates of Spain (Doadrio, 2002).
Salmo salar	**International** • Annex II and Annex V. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (European Commission, 1992). **National** • 1095/1989 Royal Decree of 8 September, in which the species hunted and fished are declared and requirements established for their protection. • 1118/1989 Royal Decree of 15 September, in which the species hunted and commercially fished are determined and requirements developed with regard to these. • Vulnerable in the Red Book of the Vertebrates of Spain (Doadrio, 2002).
Anguilla anguilla	**International** • Council Regulation (EC) 1100/2007 of 18 September 2007 establishing measures for the recovery of the stock of European eel. **National** • 1095/1989 Royal Decree of 8 September, in which the species hunted and fished are declared and requirements established for their protection. • 1118/1989 Royal Decree of 15 September, in which the species hunted and commercially fished are determined and requirements developed with regard to these. • Vulnerable in the Red Book of the Vertebrates of Spain (Doadrio, 2002).
Petromizun marinus	**International** • Annex II. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (European Commission, 1992). **National** • 1095/1989 Royal Decree of 8 September, in which the species hunted and fished are declared and requirements established for their protection. • 1118/1989 Royal Decree of 15 September, in which the species hunted and commercially fished are determined and requirements developed with regard to these. • Vulnerable in the Red Book of the Vertebrates of Spain (Doadrio, 2002). • Vulnerable in the Regional Catalog of threatened species of the vertebrate fauna of the Principality of Asturias.
Lampetra planeri	**International** • Annex II. Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora (European Commission, 1992). **National** • Unusual in the Red Book of the Vertebrates of Spain (Doadrio, 2002).

Chapter III

Mapping the temporary and perennial character of whole river networks

Chapter III: Mapping the temporary and perennial character of whole river networks

This chapter is an edited version of the research article published in the journal Water Resources Research, 53 (8), 6709-6724, by González-Ferreras, A.M. and Barquín, J. in 2017 with the title "Mapping the temporary and perennial character of whole river networks". doi: 10.1002/2017WR020390

Abstract

Knowledge of the spatial distribution of temporary and perennial river channels in a whole catchment is important for effective integrated basin management and river biodiversity conservation. However, this information is usually not available or is incomplete. In this study, we present a statistically based methodology to classify river segments from a whole river network (Deva-Cares catchment, Northern Spain) as temporary or perennial. This method is based on an a priori classification of a subset of river segments as temporary or perennial, using field surveys and aerial images, and then running Random Forest models to predict classification membership for the rest of the river network. The independent variables and the river network were derived following a computer-based geospatial simulation of riverine landscapes. The model results show high values of overall accuracy, sensitivity and specificity for the evaluation of the fitted model to the training and testing data set (≥0.9). The most important independent variables were catchment area, area occupied by broadleaf forest, minimum monthly precipitation in August, and average catchment elevation. The final map shows 7525 temporary river segments (1012.5 km) and 3731 perennial river segments (662.5 km). A subsequent validation of the mapping results using River Habitat Survey data and expert knowledge supported the validity of the proposed maps. We conclude that the proposed methodology is a valid method for mapping the limits of flow permanence that could substantially increase our understanding of the spatial links between terrestrial and aquatic interfaces, improving the research, management, and conservation of river biodiversity and functioning.

3.1 Introduction

Streams may be classified as temporary or perennial according to the permanence of their surface flow. Temporary streams are waterways that cease flowing at some point in space and time along their course (Acuña et al., 2014). Flow cessation may be caused by transmission loss, evapotranspiration, downward shifts in groundwater tables, hillslope runoff recession or freeze-up (Larned et al., 2010) and is part of the natural hydrology for streams and rivers globally (Acuña et al., 2014). Temporary streams and rivers have been defined using several terms (i.e. interrupted, intermittent, temporary, ephemeral, episodic, seasonal) according to different classifications in terms of flow, drying and periodicity (e.g. Uys & O'Keeffe, 1997). For simplicity, in this PhD Thesis we refer to all of these types of systems as temporary.

The number of studies focusing on temporary streams and rivers has increased exponentially since the 1990s (Datry et al., 2011), and there is a growing scientific interest in the ecology of temporary waterways due to their role in the water and carbon cycles. These river segments contain important links between water stored in soils, aquifers, snowpacks, glaciers and the atmosphere, and they are also important for the provision of a wide range of ecosystem services (Larned et al., 2010). Temporary streams are not only naturally widespread in dry climate areas, but they also comprise many of the first-order streams in most drainages in wetter climates (Nikolaidis et al., 2013), accounting for a significant proportion of the total number, length, and discharge volume of the world´s rivers (Tooth, 2000; Larned et al., 2010). Moreover, in the coming years, the number and length of temporary river segments and the duration and magnitude of temporary flows may increase in areas that experience drying trends due to climate change, land use alteration and water abstraction (Datry et al., 2014), which could have important consequences for river biodiversity and functioning at a catchment scale. For example, the simplification of river networks and the alteration of water fluxes have been shown to reduce the capacity of fluvial systems to recover from natural disturbances (Sabater & Tockner, 2010). The loss of perennial streams and rivers or the reduction of their lengths has large social, economic, and ecological consequences, so managers seek better ways to track and monitor the status of these systems (Turner & Richter, 2011).

The dynamic characteristics of temporary rivers present significant challenges for the assessment of ecological conditions and potentially affect the accuracy of monitoring results (Arthington et al., 2014). These challenges have not been properly addressed in some legislation, such as the Water Framework Directive (European Commission, 2000; Nikolaidis et al., 2013). Standard methods for monitoring perennial and temporary streams typically collect measurements from too few locations and do not effectively characterize the spatial extent of these terrestrial-aquatic systems.

Although a global inventory of temporary streams has not yet been compiled, several estimates exist and collectively underscore their abundance (see McDonough et al., 2011 for more information). For specific areas, the methods most used to map the spatial distribution of temporary streams are topographic maps, aerial images and field surveys (e.g. Robinson et al., 2016). However, these methods are intrinsically labor-intensive and subjective, and are not generally applicable for mapping over large areas. In recent years, these methods have been combined with various modelling techniques (e.g. Sando & Blasch, 2015) to objectively estimate temporary streams and rivers for whole river networks. Most of the previous studies that use statistical modelling to estimate temporary and perennial rivers have been performed at large regional scales where many flow gauging stations exist. These approaches are based on long flow gauging records that allow the definition of different types of temporary and perennial river flows, which are then predicted to the whole region based on specific catchment attributes (e.g. Snelder et al., 2013; Shortridge et al., 2016). However, obtaining comprehensive flow gauging records (i.e. from a sufficient number of gauges and/or time series lengths) can be very difficult or even impossible for most catchments, where flow gauges are very sparse and flow records for temporary rivers are underrepresented. This situation calls for the development of other types of approaches and data sets that facilitate the accurate estimation of the spatial distribution of temporary and perennial rivers.

Recognizing the limitations of the current digital resources and maps in representing channel extent and the degree of flow permanence (e.g. the difficulty of differentiating between temporary and perennial rivers in some forested areas using remote sensing) is important for many reasons. For example, temporary streams and rivers represent a dominant interface between terrestrial and freshwater ecosystems, and their hydrology is a critical factor influencing patterns and processes in river networks. Moreover,

accurate hydrography is a key tool for monitoring, modelling and decision making (Fritz et al., 2013). In this regard, the extent of the temporary and perennial segments in a whole river network is a basic information need for formulating appropriate strategies for biodiversity conservation. As an example, there are some recent initiatives to compile databases that integrate all the available information on the environmental characteristics and biodiversity of temporary rivers (IRBAS database; Leigh et al., 2017). For some organisms (e.g. fish), the available habitat is determined by the connectivity and extent of the perennial network. For other organisms (e.g. amphibians), the connectivity and extent of the temporary network is crucial for their survival. Temporary streams may, for example, serve as important amphibian nursery areas, because they support fewer predators than perennial streams (Reid & Ziemer, 1994). For conservation strategies and planning, it is therefore important to know the length of both perennial and temporary networks and the delineation of the boundaries between them.

Accordingly, the main goals of this paper are to develop a mapping strategy to: (1) estimate the occurrence and extent of perennial and temporary segments in a whole river network at a local catchment scale and (2) determine the main variables which play a fundamental role in determining their spatial distribution.

3.2 Methods

3.2.1 Mapping Approach

The mapping approach presented in this study comprises four steps. First, we created a Virtual Watershed with the aim of obtaining a river network for the study area (a digital representation of the surface water drainage network) that incorporates all the environmental information needed to generate the independent variables. Second, within this digital platform, we included information about the *a priori* classification of a subset of river segments (reaches of the river network) as being either temporary or perennial. This information was gathered from specially designed field surveys and aerial image data. Third, we selected several independent variables which are significant for determining the perennial/temporary character of a river segment. Fourth, we used Random Forest models to predict the temporary/perennial character of those river segments for which there was no empirical information (i.e. no field surveys or aerial

image data). The final map integrates the empirical observations with the modelled ones. All these different steps are described in detail below.

3.2.2 Virtual Watershed Approach

For this study, a Virtual Watershed was built using the Bldgrds and Netrace software packages which are contained in the 'NetMap' platform (www.terrainworks.org; Miller, 2002b; Benda et al., 2016). Virtual watersheds are computer-based geospatial simulations of riverine landscapes that include digital elevation models (DEM), synthetic hydrography, and their coupling, using a data structure to support the required analytical capabilities (for more information see Barquin et al., 2015; Benda et al., 2016). The river network (see Figure 3.1) was delineated using flow directions inferred from a 25-m DEM. To estimate the location of channel heads, we employed two criteria, one for low-gradient areas and the other applied to high-gradient areas. In the first case, channel expansion occurs primarily through fluvial processes and in the second case, channel expansion may occur via mass wasting processes. Both cases employ a slope-dependent drainage area threshold (Montgomery & Dietrich, 1992; Dietrich et al., 1993) following the equation $a_{cr}S^{\alpha} = C$, where a_{cr} is a critical specific drainage area required for channel initiation, S is the surface gradient, α is an exponent (which varies between 1 and 2), and C is a constant. The values used were $a_{cr} = 40$ m^2 (for low-gradient areas) and 300 m^2 (for high-gradient areas), $\alpha = 2$, S = 0.2 (low gradient threshold) and 0.35 (high-gradient threshold). Values of S separate channel initiation into two process domains; mass wasting and fluvial erosion of surface material. In addition to drainage-area-dependent thresholds, we required a minimum topographic convergence at the channel heads, indicated by plan-curvature values of 0.00025 or greater in low-gradient areas, and 0.01 or greater in high-gradient areas over a minimum flow length of 40 m. Physically, the C value reflects regional properties of the soil, bedrock and climate. To set threshold values that reproduce appropriate channel densities, we followed the process described in Miller (2002b) and in previous studies in the region (e.g. Benda et al., 2011). The final river network comprised 11256 river segments and set the spatial network for the integration of all the following information.

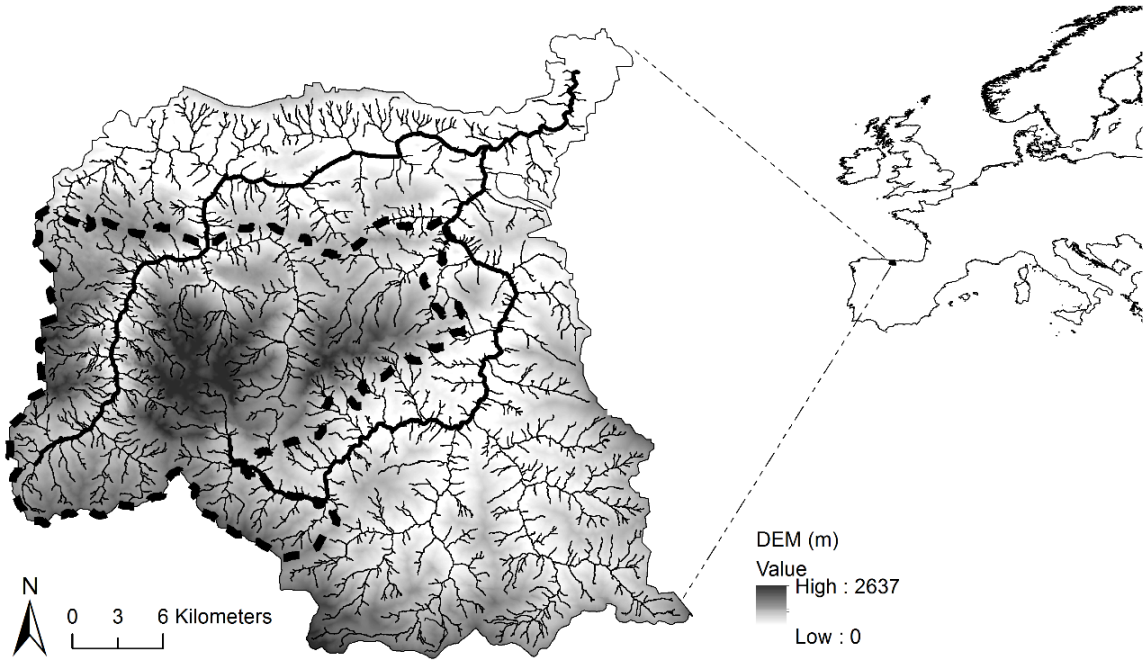

Figure 3.1. Map of the study area and representation of the Deva-Cares river network. Dashed line represents the limits of Picos de Europa National Park and black lines represent the two main axes of the river network, Deva and Cares rivers.

3.2.3 Dependent Variables

One important aspect within this study is the definition of what we considered to be temporary river segments. This definition is completely constrained by the lack of flow gauging records for the temporary river segments in the studied catchment. This lack of data prevents a clear-cut definition of the different types of temporary river segments from being made based on the frequency and duration of zero flows (e.g. Snelder et al., 2013). Instead, in this study we defined temporary river segments as those with zero flow (i.e. cessation of surface water flow, although water may be present but only in disconnected pools) during the summer (the low-flow season) in average hydrological years (see below). In contrast, we define perennial river segments to be those with perennial flow in average hydrological years. Information about the location of temporary and perennial river segments was collected from different sources and then integrated into the river network following a series of steps:

1- Field data collection. A specific field survey campaign was designed to map temporary river segments during the 2011 and 2014 summer seasons. We visited 74 and 75 different river segments, respectively (a total of 149 independent

observations) over the two seasons. We then classified each segment as being temporary or perennial according to the existence of surface flow. The summer of 2011 was considered to be a normal year according to the standardized precipitation index (SPI) developed by the Spanish Meteorological Office in its hydro-meteorological annual report (AEMET, 2016; http://www.aemet.es/es/serviciosclimaticos) while the summer of 2014 was regarded as moderately wet. Thus, we consider our mapping exercise to be a composite image of the low-flow season in average hydrological years.

2- Aerial image data. To complement the obtained field data, we visually identified temporary river segments within the rocky highlands of the Picos de Europa Karst's central massif (upstream of our surveyed tributaries and in nonsurveyed ones) using aerial images. The streams in this area do not flow during the low-flow season because of the numerous karstic ducts and the low amount of water retained in the upper catchment (see Chapter II: study area). In this area, it is relatively easy to visually identify flowing water as there is hardly any vegetation. We also complemented the field data by looking for perennial river segments in the lower parts of the catchment. In these areas the width of the river's channels is greater and the bank vegetation does not cover the segments entirely, allowing uninterrupted water flows to be identified. Aerial imagery was obtained using the Spanish National Geographic Institute's (IGN) web service of maps (WMS), and aerial images from 2011 and 2014 (with resolutions of 0.25 and 0.5 m depending on the flight) from the PNOA (National Plan of Aerial Orthophotography) project (http://www.ign.es/wms/pnoa-historico). PNOA aims to obtain digital aerial orthophotos of the entire Spanish territory, with an update period of 2-3 years and performing flights during the spring to summer period.

3- Integration of data. This is a crucial step in our methodology, as important assumptions have been made that need to be carefully considered when applying this approach to other catchments. First of all, river segments that were identified as perennial or temporary were located and labeled in the digital river network. Then we made two important assumptions: (1) we extended the temporary network upstream by assuming that all river segments upstream of a long temporary segment (> 500 m) would also be temporary and (2) we extended the perennial network downstream by assuming that all river segments downstream of a perennial segment would also be perennial. These assumptions

were made on the basis of the main hydro-geological functioning of this catchment as described in the Chapter II. Most of the tributaries in the upper parts of the karst only have water during the snowmelt season or during heavy rainfall, while most river segments in the lower valley have perennial flow maintained by a wide network of perennial springs that discharge the karst aquifer (Fernández-Giber et al., 2000; Adrados et al., 2012). Finally, two other limitations were also imposed before the modelling stages. First, this study does not include very small perennial river channels that are maintained by small spring sources in the upper part of the karst and which run dry before entering a perennial flow channel (most of them run for less than 50 m; Adrados et al., 2012). Second, this study also does not include perennial segments that run for more than 0.5 km and then go dry, as there is only one such case recorded in the catchment, associated with a karstic sink (Liordes Polje) according to Adrados et al. (2012).

3.2.4 Independent Variables

We selected a range of independent variables (Table 3.1) describing several environmental attributes that could possibly be important for determining the perennial/temporary character of the river segments in the studied area. These include topography (n = 5), climate (n = 6), land cover (n = 2) and geology (n = 4). The assignment of stream attributes to individual segments of the river network was performed using NetMap tools, and the digital information (topography, climate, land cover and geology) was summarized across a range of spatial scales, from entire catchments (drainage areas for each river segment), to adjacent hillslopes draining into individual river segments (drainage wings for each segment, referred to as segment wings). The different variables used as independent variables are described below.

Topography: catchment area, mean catchment elevation, mean catchment slope, valley floor width and drainage density were derived from the 25-m DEM using NetMap tools. Valley width was estimated from the DEM at a height of 2 times the bankfull depth elevation above the channel (for more information see: Fernandez et al., 2012).

Climate: climatic variables (Table 3.1; Climatic) were derived from monthly averages (1980-2006) calculated in a 1-km grid by interpolation of data recorded in more than

5000 weather stations of the Spanish network. These data were originally developed to be implemented into the Integrated System for Rainfall-Runoff modelling (in Spanish SIMPA model; Estrela & Quintas, 1996) by the Centre for Hydrographic Studies (CEDEX, Ministry of Public works and Ministry of Agriculture and Environment, Spain) for the assessment of water resources in natural regime at a national level.

Land cover: the area occupied by broadleaf forest and denuded areas in the upstream catchment of the river reach were derived from the Soil Occupancy Information System (SIOSE) developed by the National Geographic Institute (IGN) of the Spanish Government. This data has an appropriate scale to characterize land cover data for freshwater ecosystems (see Fernández et al., 2014 for more information).

Geology: The average rock hardness, permeability and conductivity were derived from the litostatigraphic and permeability map at scale 1:200000 developed by the Spanish Geologic and Miner Institute of the Spanish Government. These variables were calculated at catchment level using procedures described in previous studies (Snelder et al., 2008; Fernandez et al., 2012).

Although multicollinearity has no influence on the predictive performance of the model we used in this study (see below), variable importance measurements can be affected (Boulesteix et al., 2012) and the partial plots representation (see below) of the predictor-response relationship is more reliable when the predictors have low correlation (Friedman & Meulman, 2003). For this reason, and to avoid potential problems, we developed a correlation matrix (Spearman rank correlation) for the segments characterized in the previous phase, and when pairs of variables had a correlation $> |0.7|$ only one was retained for modelling (see Table 3.1 and Supplementary material 3 Figure S3.1). The variables retained were those that most reduced the total number of variables in the model (i.e. those that were correlated with a larger number of other predictors).

Table 3.1. Initial set of independent variables attributed to the river network. Bold variables are uncorrelated variables (Spearman rank correlation $\leq |0.7|$) comprised in the final set of independent variables (Figure S3.1 in the Supplementary material 3 shows the Spearman rank correlation matrix).

TYPE	CODE	DEFINITION	UNITS	Correlated with:
Topographic	**AREA_SQKM**	**Total catchment area**	**km²**	DRAIN_DEN
	MN_ELEV	**Average catchment elevation from the considered river segment to the upper most river segment in the river network**	**m**	MN_DEN, MN_EP
	MN_GRAD	**Average catchment gradient from the considered river segment to the upper most river segment in the river network**	**%**	
	VAL_FLOOR	**Width of the valley floor at 2 x bankfull depth elevations above the channel**	**m**	
	DRAIN_DEN	Drainage density. Number of segments divided by the catchment area	Nº of rivers confluences by catchment area	**AREA_SQKM**
Climatic	**MN_TEMP**	**Mean annual catchment temperature**	**ºC**	MN_maxT08
	MN_PREC	**Mean annual catchment precipitation**	**mm**	
	MN_EP	Mean annual catchment potential evapotranspiration	mm	**MN_ELEV, MN_maxE08**
	MN_minP08	**Accumulated value (average variable value from the consider river segment to the upper most river segment in the river network) for this variable: minimum value within the monthly list (1980-2006) of mean precipitation values in August**	**mm**	
	MN_maxE08	**Accumulated value (average variable value from the consider river segment to the upper most river segment in the river network) for this variable: maximum value within the monthly list (1980-2006) of mean potential evapotranspiration values in August**	**mm**	MN_EP
	MN_maxT08	Accumulated value (average variable value from the consider river segment to the upper most river segment in the river network) for this variable: maximum value within the monthly list (1980-2006) of mean temperature values in August	ºC	**MN_TEMP**

Table 3.1. (Continued)

TYPE	CODE	DEFINITION	UNITS	Correlated with:
Land cover	**MN_BLF**	**Area occupied by broadleaf forest from the considered segment to the most upper catchment point in the catchment**	**%**	
	MN_DEN	Area occupied by denuded areas from the considered segment to the most upper catchment point in the catchment	%	**MN_ELEV**
Geological	**LC_HARD**	**Average rock hardness within the segment wings**	**1-5**	
	MN_HARD	**Average rock hardness from the considered segment to the most upper catchment point in the catchment**	**1-5**	
	MN_COND	Average rock conductivity from the considered segment to the most upper catchment point in the catchment	1-5	**MN_PERM**
	MN_PERM	**Average rock permeability from the considered segment to the most upper catchment point in the catchment**	**1-5**	MN_COND

3.2.5 Modeling

We decided to use the Random Forest (RF) classification model (Breiman, 2001) with two classes (temporary and perennial). RF is a nonparametric method developed by Breiman (2001) that comprises an ensemble of individual Classification and Regression Trees (CART; Breiman et al., 1984) based on the aggregation of a large number of decision trees (a forest) from which a final prediction is averaged for all trees. RF presents a random variation by growing each tree with a bootstrap sample from the training data and using only a small random sample of the predictors to define the split at each node, where the predictions for the trees are performed using a voting system. The advantages of RF include very high classification accuracy, determination of variable importance, and the ability to model complex interactions among predictor variables (Cutler et al., 2007). Moreover, recent studies have shown that RF models predict spatial patterns in river characteristics better than other more conventional methods (e.g. Booker & Snelder, 2012). The RF technique has been previously applied in water resource studies to predict spatial patterns of different ecosystem components

such as river bed surface grain size (Snelder et al., 2011), biotic indices (Álvarez-Cabria et al., 2017) and lake trophic state (Hollister et al., 2016), among others.

RFs were developed using the R statistical language with the "caret" package, version 6.0-41 (Kuhn, 2008). We used the additional feature-selection model in caret that uses the "randomForest" (Liaw & Wiener, 2002) and "Boruta" packages (Kursa & Rudnicki, 2010). Implementing a RF model with the Boruta algorithm assists with the selection of the most relevant independent variables to include in the RF model. Boruta is a feature selection wrapper algorithm that iteratively removes the features which proved to be less relevant than random probes (Kursa & Rudnicki, 2010). Dependent-variable data was randomly partitioned into training (75%) and testing (25%) data sets, preserving the overall class distribution of perennial and temporary river segments. To fit the model, we used the cross-validation resampling method in the training set. To minimize any bias resulting from the random data splitting, a tenfold cross-validation was repeated 5 times for each of the models. This step was performed twice; first, applying the feature-selection method (Random Forest with Boruta), and then, with the final independent variables selected to be included in the modelling (the selected model). Although model performance can be optimized for the number of trees and the number of predictors used at each split, we used the recommended default values. These values were the square root of the number of predictor variables used to define the number of variables available for splitting at each tree node, and 500 as the maximum number of trees.

The average of the overall accuracy (proportion of the total number of segments that are correctly identified), sensitivity (proportion of temporary segments that are correctly identified as such), and specificity (proportion of perennial segments that are correctly identified as such) statistics was calculated for the resampling results with the optimal variables selected. These statistics were estimated according to the following equations: overall accuracy = (TP + TN)/(TP + FP + TN + FN), sensitivity = TP/(TP + FN) and specificity = TN/(TN + FP), where TP are true positives, TN are true negatives, FP are false positives and FN are false negatives. We considered the temporary class as positive and the perennial class as negative. Exact binomial 95% confidence intervals (CIs) were also calculated for overall accuracy, sensitivity and specificity (see Collett, 2002 for details). Then, the averages of the overall accuracy, sensitivity and specificity for the resampling results were chosen as model performance measures and the entire

training set was used to fit the final model. We evaluated the fitted model on the test data set and calculated the same three statistics in order to compare the results.

The importance of the independent variables was calculated according to the results of the Mean Decrease Gini Index that measures the total decrease in node impurity, averaged over all trees using the Gini Index (the purer a node is, the smaller the Gini Index is, indicating that a node contains observations which are predominantly from a single class). The Gini Index is defined as $i(t) = \sum_{i \neq j} p(i|t)p(j|t)$ where $p(i|t)$ is the probability that a case is in class i given that it is node t and $p(j|t)$ is the probability that a case is in class j given that it is node t (Breiman et al., 1984). We also used partial dependence plots to show the marginal contribution of the most important variables to the response. These plots are not a perfect representation of the effects of each variable, but they provide useful information for illustration and may be used to graphically characterize relationships between individual predictor variables and predicted probabilities of a class presence (Friedman & Meulman, 2003; Cutler et al., 2007). We used the fitted model to predict the temporary or perennial membership of segments of the river network without any empirical information on the dependent variable (i.e. without field or aerial image data). Finally, we integrated the predicted classification values with the empirical information on class membership to achieve a final map showing the temporary/perennial character of the whole river network. The maps were created with ArcGIS (ESRI, 2014). All models were developed using R 3.1.3 software (R Core Team, 2015) and the RStudio editor (RStudio, 2015).

3.2.6 Validation of Mapping Results

For an alternative validation of our final maps (which integrate the dependent variables based on empirical information and the RF model predictions), we used two external data sources. First, we used field data from River Habitat Surveys (RHS; Environment Agency, 2003) carried out in the area and, second we also garnered expert knowledge from actual forest guards in the area.

Field data were obtained from an existing database with RHS data (www.rhs.ihcantabria.com). Data were obtained from 64 500-m-long river segments during the summer seasons of 2008 (n = 42), 2009 (n = 8), 2010 (n = 1) and 2011 (n = 13). We used the information from section E of the RHS field form, where up to 10

different flow types are recorded every 50 meters (dry flow is also recorded). Because a river segment could only be classified as temporary or perennial, we considered river segments to be temporary if at least half of the spot checks were dry, otherwise they were classified as perennial. Hydrologically, the summer seasons were considered "normal" for 2008, "normal" for 2009, "moderately wet" for 2010, and "normal" for 2011, according to the standardized precipitation index developed by the Spanish Meteorological Office for the relevant months (AEMET, 2016).

To design the forest guards' validation of our mapping results, we considered the administrative organization of the Deva-Cares catchment. This catchment is divided into three different administrative regions: Castilla y León (≈150 km^2), Principado de Asturias (≈415 km^2) and Cantabria (≈640 km^2). The forest guards spent most of their days in the field up and down along the catchment because of the many duties they perform (e.g., biodiversity inventories and monitoring, enforcement of environmental regulations, issues relating to fishing and game, providing assistance to local farmers, etc.). As an example, pursuant to regional fish management policies, they annually electro-fish those parts of the river network that dry out in the summer to rescue trout that get trapped in drying pools. This means that they know the river sections that dry out each year very well. Because of their experience and knowledge of the area, we consider their opinions to be a very valuable source of data and, thus, an appropriate validation approach for the final map. In order to take advantage of their knowledge and experience and use it for our purposes, the final map with the spatial distribution of perennial and temporary segments was presented to them at meetings which were held at the headquarters of the Asturias and Cantabrian regions. During those meetings, booklets with the maps covering the headquarters' domain were handed out. These booklets were then recollected after 2-3 weeks, containing annotations made by the headquarters' personnel on the limits of the perennial/temporary character of the river network within their domain.

These results were then assigned to individual segments of the river network to perform a comparative assessment of their expert opinions, the RHS data, and the temporary/perennial characterization from our mapping results. To achieve this, we created a confusion matrix with the mapping results, the expert opinions from the forest guards and the RHS observations as reference data. Overall accuracy (proportion of the total number of river segments that are correctly identified), sensitivity (proportion of

temporary segments that are correctly identified as such) and specificity (proportion of perennial segments that are correctly identified as such) statistics were calculated for both with 95% CIs.

3.3 Results

3.3.1 Mapping Approach

A total of 2701 river segments of the river network were included in the *a priori* classification, with 1282 classified as perennial (308.5 km) and 1419 as temporary (194.5 km). Twelve independent variables were included in the final RF model with Boruta (Table 3.1), all of which were selected as relevant. Therefore, all the variables were included in the RF model to determine the temporary/perennial character of the 8555 segments without empirical information. The average of the overall accuracy (95% CIs), sensitivity (95% CIs) and specificity (95% CIs) for the resampling results were 1 (0.98-1), 1 (0.96-1) and 0.99 (0.95-1), respectively. The values of these three statistics on the test data set were 0.99 (0.98-1), 1 (0.98-1) and 0.99 (0.97-1) for overall accuracy (95% CIs), sensitivity (95% CIs) and specificity (95% CIs) respectively.

The most important independent variables (Mean Decrease Gini Index; Figure 3.2) were catchment area (AREA_SKQM), area occupied by broadleaf forest in the upstream catchment (MN_BLF), minimum monthly value of precipitation in August in the upstream catchment (MN_minP08), and average catchment elevation of the upstream catchment (MN_ELEV). Conversely, the variable with the least importance in the model was the average rock hardness within the segment wings (LC_HARD). Partial plots of the most influential variables (Figure 3.3) indicate that as watershed area decreases, there is a higher probability of a temporary classification (Figure 3.3a). Conversely, as watershed area increases, there is a higher probability of a perennial classification with an important threshold at approximately 20 km^2. In the case of MN_BLF, the probability of temporary classification decreases as the percentage of forest in the catchment increases (until ≈20%). For MN_BLF values between ≈20% and 70%, the probability of temporary segments is low and more or less constant, but doubles for MN_BLF values above 70% (Figure 3.3b). For the other two most important variables (MN_minP08 and MN_ELEV; Figure 3.3c and Figure 3.3d), the probability of perennial classification is higher for their median values, and in the case

of MN_minP08, when these variable approaches zero (see Figure 3.4 for the distribution of temporary/perennial character recorded in the empirical observations).

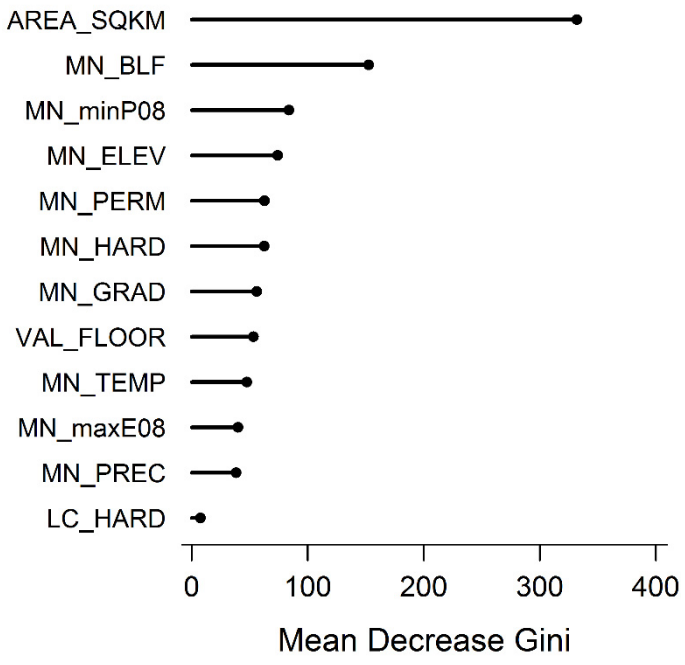

Figure 3.2. Importance of the independent variables (see variable code description in Table 3.1) in the fitted model in relation to the Mean Decrease Gini Index.

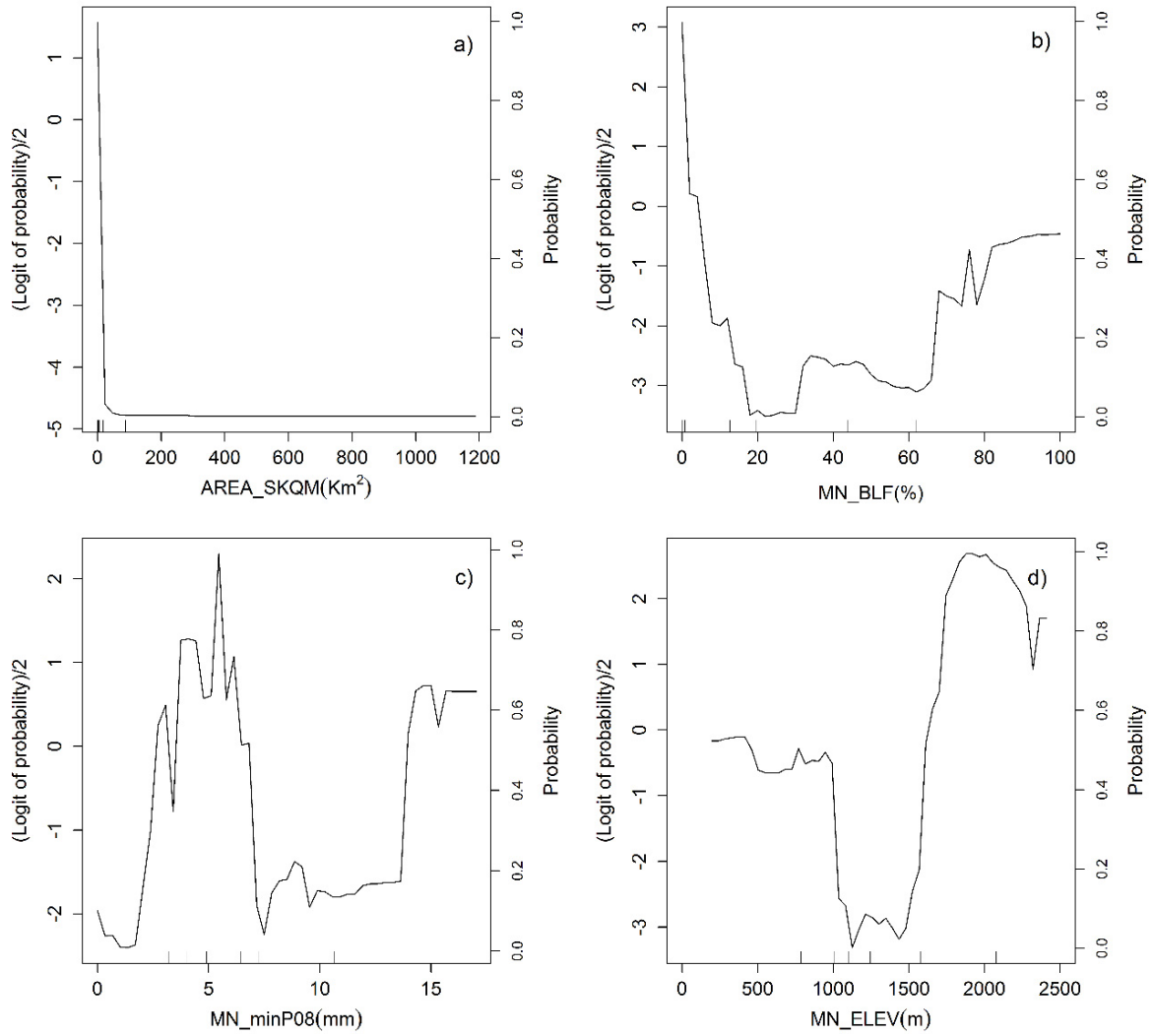

Figure 3.3. Partial dependence plots for the 4 most influential variables in the model for the temporary class (a: AREA_SQKM; b: MN_BLF; c: MN_minP08; d: MN_ELEV: see variable code description in Table 3.1). The "rug" at the bottom show the deciles of the distribution of sites across that independent variable. In the case of the perennial class, partial dependent plot is the mirror image of these partial dependence plots, and only one class was used for interpretation. The values of the left y-axis (logit of probability/2) are also represented in probabilities on the right y-axis.

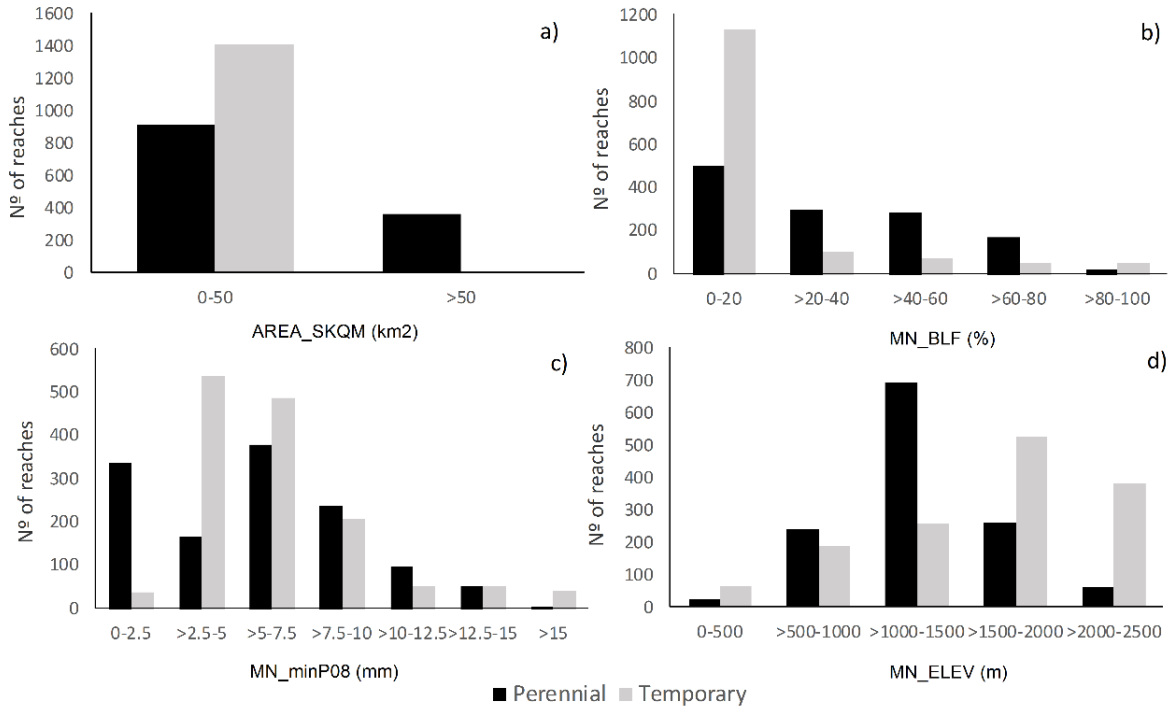

Figure 3.4. Distribution of the temporary and perennial character in the 2701 segments used as dependent variables according to different ranges of the 4 most important independent variables in the Random Forest model (a: AREA_SQKM; b: MN_BLF; c: MN_minP08; d: MN_ELEV: see variable code description in Table 3.1).

The fitted model predicted 6106 (818 km) river segments to be temporary, while 2449 (354 km) were regarded as perennial (Figure 3.5b). The final map of the Deva-Cares catchment in low-flow conditions (Figure 3.5c) comprises the predicted river segments (Figure 3.5b) and the river segments with initial *a priori* classification data (Figure 3.5a). This final map shows 7525 temporary river segments (1012.5 km) and 3731 perennial river segments (662.5 km).

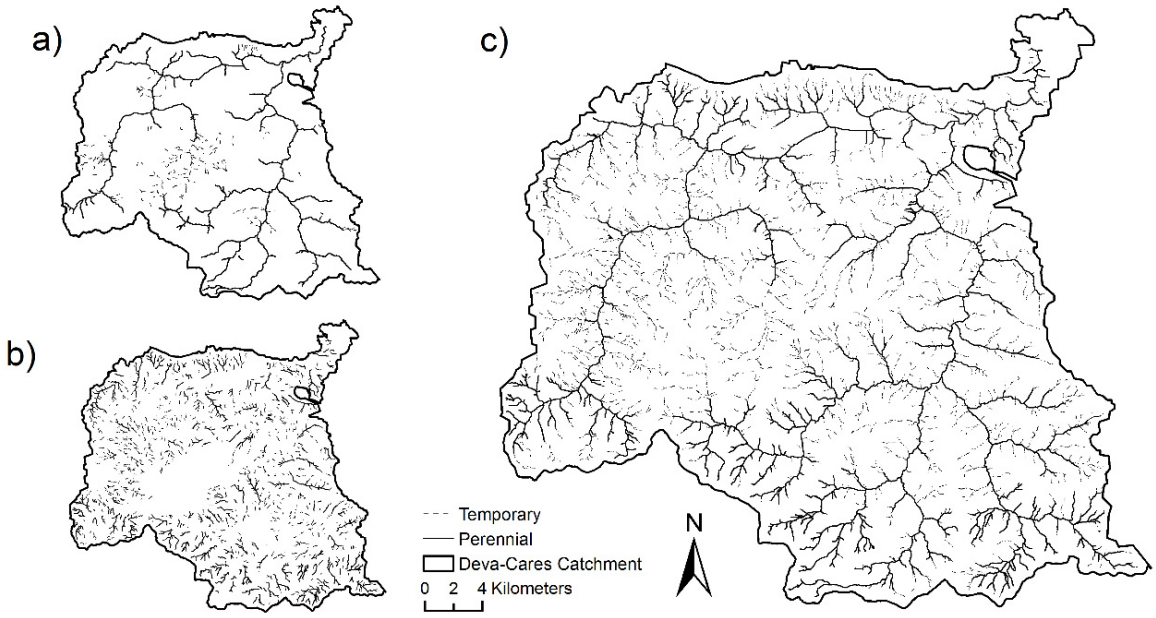

Figure 3.5. a) Spatial distribution of the segments whose class membership was assigned by aerial images or field measurements. b) Spatial distribution of the segments whose class membership was predicted with Random Forest model. c) Representation of the temporary and perennial segments in the entire river network comprising the segments predicted (modelled with Random Forest) and segments used as dependent variables (assigned by aerial images or field measurements).

3.3.2 Validation of Mapping Results

The validation of the perennial/temporary characterization of the mapping results (Figure 3.5c) with RHS data (64 segments with RHS surveys data) shows the following values of overall accuracy = 0.91 (0.81-0.96), sensitivity = 0.67 (0.09-0.99) and specificity = 0.92 (0.82-0.97).

Regarding the evaluation of the expert opinions of the forest guards, the evaluation from Principado de Asturias could not be quantitatively assessed, because they did not provide geographical indications on the map. However, their general comments were that they agreed with the map, although suggesting that the highest perennial tributaries located in the Picos de Europa National Park should be slightly trimmed down. Conversely, the Cantabrian forest guards provided geographical indications for their entire respective area (5623 river segments). The evaluation of the Cantabrian forest guards shows high values of overall accuracy = 0.9 (0.9-0.91), sensitivity = 0.9 (0.89-0.91) and specificity = 0.91 (0.9-0.92).

3.4 Discusion

The strategy presented in this study to map the perennial/temporary character of river segments for a whole river network has been shown to be a plausible approach from a statistical point of view, and from the perspective of experts in the field. The length of the temporary river segments (1012.5 km) in our study area represented around 60% of the total channel length (1675 km) of the river network, so temporary streams should not be neglected when conducting catchment scale studies. Similar percentages have been found in other studies. For example, Nadeau and Rains (2007) reports that 59% of the total stream length in the USA, excluding Alaska, are temporary streams. Tzoraki et al. (2007) reports that 43% of the total area of Greece is drained by temporary rivers. We believe that the maps generated in this study could be a key digital resource for future research and management strategies in the selected catchment, with numerous potential applications.

3.4.1 Mapping Approach

Field surveys and aerial images provide information that is relatively easy to collect in order to classify rivers as temporal or perennial together with a modelling approach. Other studies have used other sources of data, such as the involvement of citizens (e.g. Turner & Richter, 2011; Datry et al., 2016) and gauging stations (e.g. Snelder et al., 2013). Gauging stations provide information about the frequency and duration of temporary flow using flow time series, and facilitate a more specific definition of flow intermittency. However, most catchments typically have few gauging stations, and these are usually located in perennial river segments. This was the case in our study area, in which there were few active official gauging stations (three in river channels and four in reservoirs) all of which were situated in perennial river segments.

In this study, modelling the temporary and perennial character of river segments yielded high values of overall accuracy, sensitivity and specificity in the evaluation of the fitted model on the training and testing data set. These high values demonstrate the model's capacity to learn from the training data set and to identify the main relationships between the dependent and independent variables, and indicates the prediction capacity of the model. Conversely, Snelder et al. (2013) reported that RF performed poorly at classifying flow regimes, and suggested that the performance results were due to the

fact that intermittence is also controlled by processes acting at smaller scales. We believe that the good performance of the model in our case might be related to a number of different issues. First, the hydrogeological functioning of the Deva-Cares catchment allowed us to make some assumptions (section 3.2.3; i.e. considering any segment upstream of a segment empirically classified as temporary to also be temporary, and any segment downstream of a segment empirically classified as perennial to also be perennial) that may have improved the performance of some of the selected predictor variables. In this regard, the catchment area played an important role in differentiating between temporal and perennial river segments, with 20 km^2 being an important catchment size threshold. However, these assumptions might not hold in other catchments where different hydrological processes dominate, for example in large rivers where alluvial aquifers generate temporary flows (e.g. Larned et al., 2011), or where anthropogenic water regulation changes the temporary/perennial character of a river segment (e.g. water abstraction or diversion; (Datry et al., 2014)). In these cases, it might also be necessary to include other variables related to groundwater dynamics, such as riverbed permeability or aquifer structure (Snelder et al., 2013) or anthropogenic variables such us the distance to the point of abstraction. Second, our modelling approach was developed in a local catchment in which very small tributaries were considered (employing a slope-dependent drainage area threshold for estimating the location of channel heads in the delineation of the river network), but limitations to identifying temporary/perennial segments had been previously identified using different criteria (e.g., permanent channel length criteria). These assumptions might also be inappropriate when working at very large regional scales where most of the headwaters have been trimmed off from the river network (e.g. establishing a minimum area or order) and local knowledge regarding the abundance and location of special cases (e.g. catchments with numerous sinkholes) is not available (e.g. Snelder et al., 2013). Third, the use of aerial images in the current study was possible because of the lack of woody vegetation at high altitudes and the large channel width of the lower segments. These characteristics allowed us to use the aerial images to identify temporary and perennial segments, respectively, in the river network. In other locations with different characteristics, the use of unmanned aerial vehicles (UAVs) might be an appropriate approach to cover large parts of the river network that could be used as segments in the training data set. While the use of UAVs for hydrological processes and modelling is still experimental and in continuous development, some studies have already shown

satisfactory results (Spence & Mengistu, 2016). Finally, our training data set included a balanced training data set (47.5% perennial and 52.5% temporary), which has been shown to be very relevant for increasing the accuracy of RF models (González-Ferreras et al., 2016). Conversely, extremely imbalanced classes result in poor accuracy for the minority class because RF tends to focus more on the prediction accuracy of the majority class (Chen et al., 2004).

In relation to the independent variables, the catchment area and broadleaf-forest-percentage cover in the upstream catchment were the most important variables for classifying a segment as temporary or perennial, together with the minimum monthly value of precipitation in August, and the average catchment elevation. Catchment area has been shown to be an important determinant of perennial and temporary channels in other studies (Svec et al., 2005; Snelder et al., 2013). The high frequency of temporary river segments in small catchment areas is supported by the temporary/perennial character distribution obtained from the empirical observations (Figure 3.4a).

The second most important variable, forest cover in the catchment, showed similar responses at either extreme of its continuum. It is known that different types of forest can have an important role in hydrology (Cui et al., 2012). However, within this study we only considered broadleaf forest, because other types of forests were only present in 1% of the river segments with very low cover values. Minimum forest cover values tend to correlate with temporary character occurring more frequently (Figure 3.4b). These results match with the high occurrence of temporary river segments in the alpine area (Figure 3.4b). In the Deva-Cares catchment, temporary segments are common in the alpine karst areas where mountain grasslands and denuded rocks dominate and, consequently, a low percentage of forest is found. At the other extreme, the probability of temporary river segments also increased with higher values of forest cover. This result is consistent with studies that find increased water yields following timber harvesting (e.g. Smerdon et al., 2009). In our case, this result corresponds to small catchments ($\leq$5 km^2) where broadleaf forests have been preserved (i.e. a high percentage of cover). Catchment forest coverage has also been shown to play a key role in determining hydrological spatial patterns in other studies (e.g. Cui et al., 2012). Moreover, other variables related to forest presence could also play important roles in determining the temporary or perennial character of small river segments. For example,

Belmar et al. (2016) has shown that mature forests in Cantabrian catchments may provide higher base flows during the summer months compared to young forests.

3.4.2 Validation of Mapping Results

The validation of our results using RHS survey data and expert knowledge from forest guards supports the methodology proposed in this work for mapping the temporal and perennial character of river segments. Both validations have shown high values of overall accuracy (≥0.9). In the specific case of RHS data, the proportion of perennial segments correctly identified was high (specificity = 0.92), but the proportion of temporary segments correctly identified presented lower values (sensitivity = 0.67). This sensitivity value could be related to the lower proportion of temporary segments (n = 3) within the RHS data set (n = 64). This could negatively influence the validation process due to an unbalanced class data set or a dearth of information caused by a small sample size (Ali et al., 2015). For this reason, including a larger number of temporary segments within the RHS data set would provide for a more robust validation process. When using the expert opinion data, the quantitative evaluation showed high values of sensitivity and specificity (0.9 and 0.91 respectively) in the Cantabrian region, which represents more than 50% of the study area. The qualitative assessment of the other administrative area (Principado de Asturias) was also positive. Regarding the model and the independent validations, we consider that the final map results represent a major improvement of the digital cartography of temporary and perennial river channels in the study area. The length of the segments using our mapping approach is greater than in existing maps. Our map has 1675 km of river segments, entailing 1012.5 km of temporary rivers and 662.5 km of perennial rivers, while the most detailed existing map of the area (IGN, 2016; National Topographical Base of Spain at scale 1: 25000 – BTN25) has 1126 km of river channels, listing 845 km of temporary rivers and 280 km of perennial rivers. Other studies have shown that this kind of topographic map also underestimates the total length of river channels (e.g. W. F. Hansen, 2001). Moreover, comparing this map with our results, a great part of the length of the temporary rivers in the BTN25 (≈ 40%) are actually perennial according to our map, while less than 15% of the total length of perennial rivers in the BTN25 are temporary according to our mapping approach. Thus, our results extend and improve the existing information, highlighting the need to update the current information.

The knowledge gathered from the forest guards from the Cantabrian region allowed us to identify sinkholes in the area, which were not taken into account in the *a priori* classification which was based on our assumptions. This situation comprised six zones, where perennial flows exist upstream of temporary segments. The total length of temporary river segments downstream of perennial flows is 3.4 km, and the total length of perennial river segments upstream of temporary segments is 16.3 km. These values support our initial assumptions, because their length in relation to the whole river network length (temporary and perennial) in the Cantabria part of the catchment (850.5 km) is minimal. However, the identification of these areas, especially in catchments where such areas are significantly represented, is important for the continuity of river processes and has a major impact on river biodiversity and functioning patterns. Accordingly, we stress the need to gather this type of punctual information in future studies whenever possible.

3.4.3 Potential applications

Mapping the temporary or perennial character of a river network can provide a better understanding of hydrologic systems and the interaction between terrestrial and aquatic interfaces at large spatial scales (i.e. landscapes or watersheds). The current lack of data on how temporary and perennial river segments are spatially organized in river networks hinders the development of regional applications. To compensate, upscaling methods are needed to extrapolate information from river segments to whole catchments (Blöschl, 2006). In our study, the combined use of field data, aerial images and modelling provides a simple and replicable methodology to upscale information about perennial and temporary river channel character to the whole river network. This simple representation of the limits between temporary and perennial river segments for whole river networks at a catchment scale constitutes a first step in various applications. This exercise estimates the extent and magnitude of each class in the catchment and can stimulate research and management for a variety of different objectives. Below, we describe the importance of some of these applications to illustrate the relevance of this exercise:

1) Monitoring of areas likely to experience changes due to global change. Some evidence suggests that climate-driven temporary flow has increased and that it will continue to increase in the future (Larned et al., 2010). It is therefore important to

delineate the potentially most sensitive zones that might suffer from the effects of global change. RF models can provide class probability results to help to delineate these zones by mapping the probability of a segment's being temporary or perennial instead of using the hard binary classification (temporary/perennial). In our study area, for example, we ran RF probability class and around 130 km of channels have a temporary class probability between 0.45 and 0.55. All of them are located in catchments with areas less than 8.5 km^2 and could be identified as potentially most sensitive or transition zones.

2) Delimit terrestrial and aquatic systems and locate their interfaces. Because temporary streams are hydrologically dynamic, providing both terrestrial and aquatic habitats, their characterization and delimitation is important for determining different interactions, such as exchanges between terrestrial and aquatic organic matter and biotic interactions between terrestrial and aquatic organisms (Datry et al., 2011). Moreover, temporary channels function differently than perennial ones with respect to biogeochemical fluxes (Datry et al., 2014) and may have different impacts on carbon and nutrient fluxes. Therefore, it is important to identify the transitions between aquatic and terrestrial phases, referred to as hot spots and hot moments for biogeochemistry (McClain et al., 2003).

3) Habitat delineation for different groups of organisms. In temporary streams that desiccate entirely, fish must seek refuge in perennial segments, whereas in streams that dry partially, some fish can survive in disconnected pools (e.g. Pires et al., 1999). Considering that stream drying is stressful for fish and causes high mortality (Davey & Kelly, 2007), fishes are generally restricted to perennially flowing segments. In the case of amphibians, temporary streams are important, because the differential vulnerability of larvae to aquatic predators may exclude some species from perennial streams (Woodward, 1983). The temporary/perennial character of a channel has been shown to be important for invertebrates (e.g. Leigh et al., 2016) and algae (e.g. Robson & Matthews, 2004). The spatial distribution of temporary and perennial channels has important implications for the distribution patterns of aquatic species and is thus a key feature for aquatic biodiversity management and conservation.

3.5 Conclusions

This study proposes a relatively easy method for estimating the occurrence and extent of perennial and temporary segments in whole river networks at catchment scale. This strategy allows the determination of which variables are the most important based on catchment characteristics which play a fundamental role in determining the spatial distribution of flow permanence. Our approach can be applied anywhere in the world using minimal data resources, although the applicability of our assumptions to other locations should be carefully considered and be based on a working knowledge of the fundamental hydrological processes of the studied catchment. Thus, with a few field surveys taken in the low-flow season, access to aerial images and a virtual watershed approach (Benda et al., 2016), it is possible to obtain dependent and independent variables to build a temporal and perennial river segment classification model. Incorporating available knowledge from locals and experts in the area can also enhance the mapping approach and improve the final digital map representing the spatial distribution of temporary and perennial segments in the particular river network.

3.6 Supplementary material 3

This Supplementary material contains the Figure S3.1 referred to in the main text of the Chapter III.

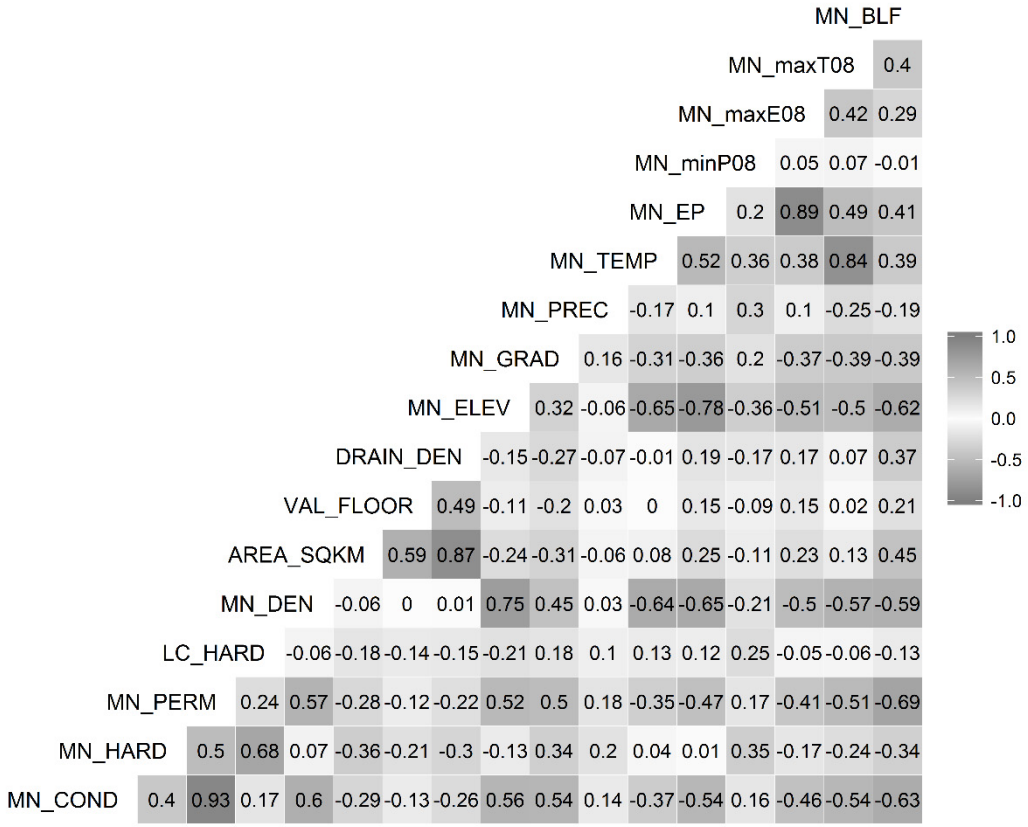

Figure S3.1. Spearman rank correlation matrix of the 17 potential independent variables (see variable code description in Table 3.1 in the main text of the Chapter III). Positive values indicate positive correlation and negative values indicate negative correlation.

Chapter IV

Spatial variability of *Salmo trutta* at a river network scale. What variables are influencing spatial distribution of population density?

Chapter IV: Spatial variability of *Salmo trutta* at a river network scale. What variables are influencing spatial distribution of population density?

This study, performed by González-Ferreras, A.M, Alonso, C. and Barquín, J, is under preparation to be submitted for publication in a SCI journal.

Abstract

Identifying spatial variability on biological populations and the factors determining them in a whole river network is paramount to increase our understanding on river patterns and processes. The dispersal of fish species can only occur within the river, thus, aquatic habitat characteristics and longitudinal connectivity appear as key factors that might influence fish spatial distribution patterns. However, information on these key variables at a network scale is usually not available or the type and spatial scales of environmental variables is limited. In this study, we explore the role that habitat variables at different spatial scales (catchment, segment and reach) are playing on determining spatial density patterns of brown trout for each age-class (young-of-the-year, juvenile and adult) at a whole river network scale (Deva-Cares river, Northern Spain). We also explored the role of hydrological and Euclidean distance, and the presence of impermeable barriers on explaining brown trout spatial density patterns. The methodology used is based on a selection of environmental variables with influence on fish density data through a correlation analysis and the use of Generalized Linear Models to analyse the relation of the environmental variables with fish density. Lastly, we used a Mantel test and partial Mantel test to assess for patterns in the distribution of the densities across field sites. The GLM model for young-of-the-year (0+) explained 25% of the deviance with 3 significant variables ($p < 0.05$), each corresponding to one of the three spatial sales: adult brown trout density, total catchment area and mean annual temperature within the segment wings. In the case of juvenile (1+) only mean bankfull width was significant, explaining 30% of the deviance. The deviance explained for adult (2+) was 24% with one catchment variables (area occupied by denuded areas), one segment variable (active channel width) and two reach variables (area occupied by

broadleaf forest within a 200 m buffer along the surveyed river reach and mean water velocity) selected. Simple and partial Mantel test revealed significant correlation between brown trout densities and presence of impermeable barriers and between brown trout densities and environmental data for all age classes, while hydrological distance was only significant for young-of-the-year. We conclude that population density is age-structured spatially, and that niche and dispersal are both important factors influencing the spatial variability of brown trout density at a river network scale. The higher mobility of older age-classes increases the importance of the dispersal-connectivity relation, while niche is more important in earlier, less mobile, life stages. The combination of both factors explains a greater proportion of the brown trout spatial density patterns.

4.1 Introduction

Rivers are ecosystems hierarchically organized and spatially nested, ranging from catchment and streams to aquatic microhabitats, where the higher scale constrains the lower scale and therefore microscale habitat patterns are constrained by macroscale patterns (Frissell et al., 1986). Habitats and freshwater organisms are potentially connected along longitudinal, lateral and vertical spatial dimensions (Ward, 1989). In turn, organisms than inhabit river systems are constrained by the branching structure depending on their mode of dispersal (Tonkin et al., 2018). Some organism are subject to in-stream dispersal (dispersing actively through swimming, like some fish, or passively through drift, like some freshwater insect larval stages; e.g. Bilton et al., 2001; Davey & Kelly, 2007) while other organisms disperse overland (e.g. some adullt stages of freshwater insects; Chaput-Bardy et al., 2008). The former species are likely to be more influenced by network structure (Tonkin et al., 2018). Moreover, dispersal depends on dispersal traits of organisms and physical structure of networks highlighting the connectivity-dispersal relation (Tonkin et al., 2018) that may influence the spatial patterns of biodiversity (Altermatt & Fronhofer, 2017). Even for single species, connectivity requirements may vary among life stages due to their different dispersal capabilities (Fullerton et al., 2010). For example, amphibians can present different dispersal behavior for the different age-classes: egg, tadpole, metamorph, juvenile and adult (Vimercati et al., 2017). It should be noted that the loss of longitudinal connectivity (e.g. presence of cross-sectional barriers) constitute one of the largest

threats to river ecosystems (Vörösmarty et al., 2010) disturbing connectivity-dispersal relations and fragmenting the habitat.

In addition to the dispersal, another major factor that may also influence the spatial distribution and persistence of fish species are the biotic and abiotic environmental factors, which are organized at different spatial scales. Most of the studies that analyze this relationship have been focused mainly in one of two scales of environmental variables: *local* (e.g. river reach variables; Terra et al., 2016) *vs regional* (e.g. catchment variables; Kelso & Minns, 1996). However, not many studies have analyzed the effect of habitat characteristics on spatial population variability at several different spatial scales for a whole river network. This multi-scale approach is needed for understanding fish and their habitat at the riverscape scale at which many processes critical to populations and communities occur (Fausch et al., 2002).

Species distribution patterns (by dispersal or biotic and abiotic environmental factors) has been explained in community ecology by two theories: neutral theory and niche theory, which can be located at the extremes of a continuum (Gravel et al., 2006). Niche theory assumes that species are fundamentally different and these differences allow them to coexist because of the separation of their niches (Hutchinson, 1959; Wennekes et al., 2012). Neutral theory assume that species are functionally identical and diversity is explained as a stochastic balance between speciation and extinction (large scale) or immigration and extinction (small scale; (Hubbell, 2001, 2005)). Previous studies indicate that both neutral and niche theories are structuring communities, in some cases niche is more important (Rodrigues & Lima-Ribeiro, 2018), while in other cases dispersal is more important or even both might be important on determining species distribution patterns (Thompson & Townsend, 2006). Both theories have tried to be integrated through the concept of metacommunity, which is defined as a set of local communities that are linked by dispersal (Leibold et al., 2004; Altermatt, 2013). There are four paradigms in metacommunity theory: species sorting, mass effects, neutral dynamics and patch dynamics. These paradigms differ in the role of local (species interaction and local environmental conditions) and regional (dispersal) processes in community assembly (see Leibold et al., 2004; and Altermatt, 2013 for more information). According to Brown and Swan (2010), these paradigms can act simultaneously in a riverine metacommunity depending on local and regional process (i.e. species sorting in headwaters and mass effects in mainstream). Metacommunity

theory has been influenced by metapopulation concept, defined this last as a set of local populations within some larger area, where typically migration from one local population to at least some other patches is possible (*sensu* Hanski & Simberloff, 1997). Metapopulation is categorized in four types in terms of the different roles that imply local extinction: mainland-island and source-sink, patchy, non-equilibrium and classical (see Harrison, 1991 for more information)

Among aquatic organisms, fish are one of the most interesting groups to understand biological-environment and connectivity-dispersal associations and test the aforementioned theories. This group may exhibit specific spatial patterns and different habitat preferences (Schlosser, 1991) which can be influenced by density-dependent and/or density-independent processes (Milner et al., 2003) and its dispersal is strictly restricted to the river network. Salmonids represent an interesting group to study these relations. Currently there is some debate about whether these populations are or not metapopulations (Rieman & Dunham, 2000; Schtickzelle & Quinn, 2007) and the influence of both habitat and dispersal in the occurrence and persistence of salmonids populations (Cooper & Mangel, 1999). Moreover, several perturbations as global warming (Almodovar et al., 2012) or habitat fragmentation (Carlos Garcia de Leaniz, 2008) are altering these populations around the world turning them into target species for management and conservation policies. Among them, brown trout (*Salmo trutta* Linnaeus, 1758) is one of the most widespread salmonid species in European inland waters and its natural distribution is fundamentally restricted to this area, although it has been introduced in many countries worldwide (Elliott, 1989c). Moreover, brown trout is of great importance from an ecological and socio-economic point of view, as it is the fish top-predator in many freshwater ecosystems (Jensen et al., 2008; Sánchez-Hernández, 2016) and one of the most popular and important sport-fishing species (Almodovar & Nicola, 1998). Previous studies indicates that spatial niche selection by brown trout is size structured (Heggenes et al., 1999; Ayllón et al., 2010) and some of the most important environmental variables for the species are water depth, velocity, substrate and cover (Heggenes et al., 1999). Young individuals are associated with shallow areas and slower water while adult individuals are associated with deeper areas (Heggenes et al., 2002; Klemetsen et al., 2003). Dispersal patterns of brown trout have been reported to be highly variable, since population may be composed of both stationary and mobile individuals (e. g. Bridcut & Giller, 1993), being the mobile

fraction less abundant (Young et al., 2010). In relation to the different age-classes, dispersal of young-of-the-year brown trout is limited (Vatland & Caudron, 2015)**,** while adults usually have the highest dispersal rates (Olsson & Greenberg, 2004). Because dispersal can only occur along the stream channel in an up- or downstream direction, loss of longitudinal connectivity by barriers and the loss of suitable habitat may influence the spatial patterns of brown trout, thus originating spatial isolation, population fragmentation or decreased individual fitness (Gosset et al., 2006).

Despite the numerous studies focusing on brown trout populations, there are not many considering both brown trout populations and environmental variables at different spatial scales for a whole river network, besides considering the age-structure of the population and the presence of all the longitudinal barriers in the catchment. Thus, the main goals of this paper are i) to explore and quantify which environmental variables at different spatial scales (catchment, segment and reach) are playing a more important role on determining the density of the brown trout for each age-class and ii) to find out whether hydrological and Euclidean distances and presence of impermeable barriers are important on determining brown trout spatial distributional patterns. Our initial hypothesis based on the importance of the niche theory is that environmental variables will influence brown trout density for all age classes. Moreover, reach variables will be more important determining fish density because the correlation strength would decrease with increasing in scale (Johnson & Goedkoop, 2002), although the specific variables will be different for each age class depending on their habitat preferences (Klemetsen et al., 2003). Regarding the dispersal-connectivity relationship, we hypothesized that connectivity and therefore the presence of impermeable barriers will be only important for the age classes with more dispersal capacity (juvenile and adults). Euclidean distances *a priori* will not be important for any of the brown trout age classes because they do not disperse overland, while hydrological distance will be important for all age-classes. Therefore, we predict that environmental variables or number of reproducers will have more influence on young-of-the-year density because of their low dispersal, while both environmental variables and connectivity might be both important for determining juvenile and adult age-classes densities (see Figure 4.1)

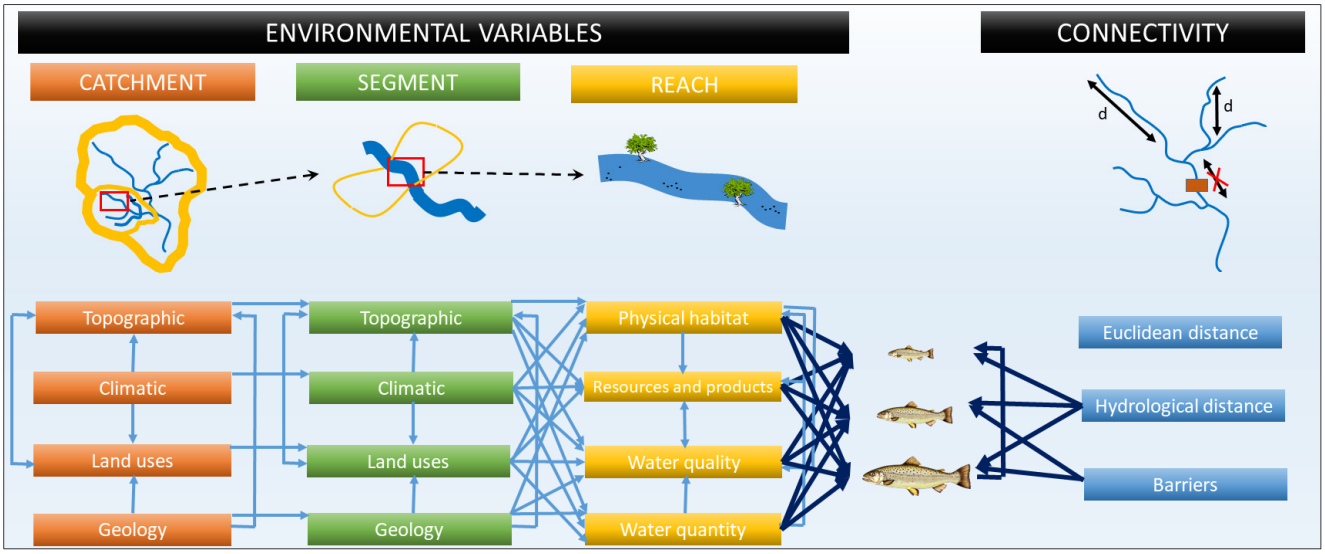

Figure 4.1. Graphic representation of the studied hypothesis. Groups of environmental variables hierarchically organized at catchment, segment and reach scale and hypothetical relations between groups of variables and brown trout density age-classes (left side). Hypothetical relations between Euclidean distance, hydrological distance and presence of barriers with brown trout density age-classes are also shown (right side).

4.2 Methods

4.2.1 Data

4.2.1.1 River network

Headwater, temporary river reaches were assumed to be only sporadically inhabited by brown trout and, thus, they were not considered any further in the study. Moreover, there were no major temporary river reaches in the middle part of the river network. For this reason, the river network used in this study covers only the perennial river reaches of the Deva-Cares catchment in low-flow conditions (for more information see González-Ferreras & Barquín, 2017 and Chapter III). The perennial river network (see Figure 4.2) obtained by means of the Virtual Watershed and mapping approaches (Chapter III) is composed of segments with lengths ranging from 30 to 805 m and set the spatial digital network for the integration of all the environmental information (see below). The river network contains 139 longitudinal barriers (see Figure 4.2), being 102 of anthropogenic origin and 37 natural.

4.2.1.2 Fish data

The fish data set was obtained from 76 field sites sampled between July and October 2014 (low-flow conditions) using electrofishing techniques (see Figure 4.2). All the field sites were located in the Deva-Cares catchment and were selected for their distribution along the fluvial network, covering a broad spatial sample from the headwater to the mouth to represent the spatial variability of the species. Each sampling was carried out on a minimum area that included a sequence of mesohabitats that was representative of the river reach. In the present study, only the first capture data were used with the objective of homogenizing the data and to keep the data comparable among sites. A portable electric fishing device (power 1.3 kW) was used, with direct current generation of 300 to 500 V, or pulsating up to 940 V with variable frequency (25 to 100 Hz). Natural barriers or nets were used to block upstream and downstream each field site. Captured brown trout individuals were sedated with eugenol, counted, weighted and their furcal length measured. Fish were placed into holding boxes to recover and then returned alive to the stream near the point of capture. Individuals were classified into three age-classes (young-of-the-year "0+", juvenile "1+" and adult "2+") by means of a visual analysis based on the frequency distribution of the fork length (Petersen, 1986). Fish sampling sites were located and labeled in the digital river network.

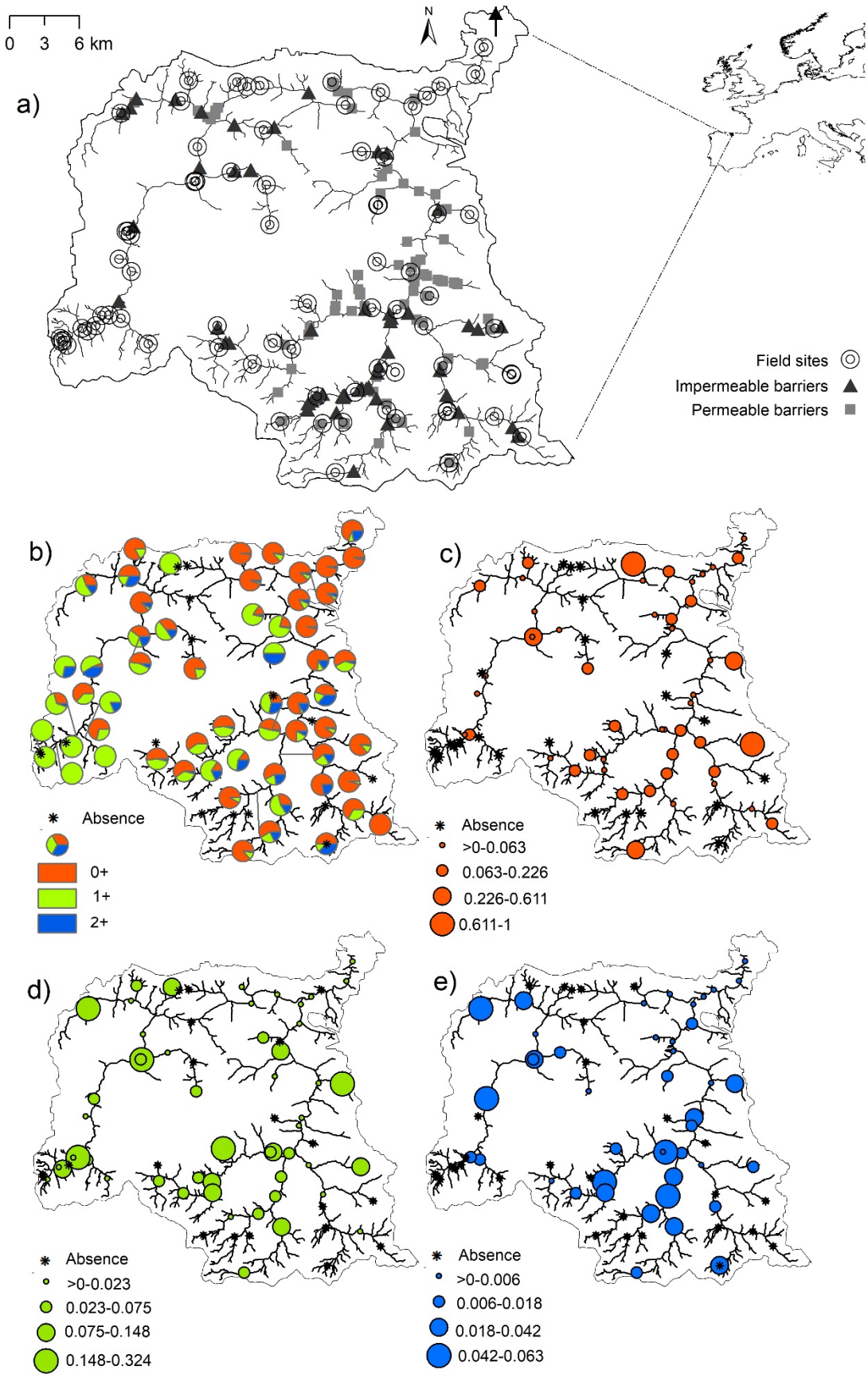

Figure 4.2. a) Map of the study area and representation of the Deva-Cares river network. Field sites from the fish data sets and longitudinal barriers (permeable and impermeable) are also

show. b) Distribution (%) of *Salmo trutta* age-classes in each field point. c) Density of *Salmo trutta* 0+ (ind/m^2) in each field point represented by categories (Jenks natural breaks classification). d) Density of *Salmo trutta* 1+ (ind/m^2) in each field point represented by categories (Jenks natural breaks classification). e) Density of *Salmo trutta* 2+ (ind/m^2) in each field point represented by categories (Jenks natural breaks classification).

4.2.1.3 Environmental variables

We selected a range of environmental variables (see Table S4A.1 in the Supplementary material 4A) that could potentially explain the spatial variability of each age-class brown trout density at three different spatial scales: reach (river reach itself), segment (adjacent hillslopes directly draining into a river reach) and catchment (upstream catchment area draining into a river reach). Selected variables included topography (n = 13), climate (n = 8), land uses (n = 7), geology (n = 6), water quantity (n = 1), water quality (n = 13), physical habitat (n = 16) and resources (n = 8). Many of the selected variables have been considered important for brown trout populations in previous studies (B. Jonsson & Jonsson, 2011; Lobón-Cerviá & Sanz, 2017). A detailed explanation of how each environmental variable has been measured can be found in the Supplementary material 4A, here we only include a brief description for each group of variables.

-Topographic: variables were derived from a 25-m DEM using the NetMap platform (Miller, 2002b; Benda et al., 2016; www.terrainworks.org).

-Climatic: variables were derived from monthly averages (1980-2006) calculated in a 1-km grid by interpolation of data recorded in weather stations of the Spanish network. These data were originally developed to be implemented into the Integrated System for Rainfall-Runoff modelling (in Spanish SIMPA model; Estrela & Quintas, 1996) for the assessment of water resources in natural regime at a national level.

-Land uses: variables were derived from the Soil Occupancy Information System (SIOSE; IGN, 2011).

-Geological: variables were derived from the lithostatigraphic and permeability map at scale 1:200000 developed by the Spanish Geologic and Mine Institute (IGME, 2006).

The previous variables were derived from available GIS layers, while all the variables defined below were surveyed in the river reaches.

-Water quantity: flow was obtained from a single cross-section profile data per site measured by means of a portable Acoustic Doppler Velocimeter (FlowTracker Handheld Acoustic Doppler Velocimeter, SonTek/YSI Inc., United States) and using the mid-section discharge equation.

-Water quality: water temperature variables were obtained by means of loggers (HOBO® model UA-002 temperature/Light data logger, Onset Computer Corporation, Cape Cod, MA, USA or Keller Series 36XW). Conductivity and pH were measured in situ with an YSI 556 Multi-Parameter Handheld Meter (YSI Inc., Yellow Springs, OH, USA) and the rest of variables were measured in the laboratory from a water sample preserved in 250 mL polyethylene containers on ice and transported to the laboratory. All the physicochemical characteristics of water were estimated according to Standard Methods for the Examination of Water and Wastewater (APHA. et al., 1999).

-Physical habitat: barriers were qualified permeable or impermeable for brown trout species according to the physical characteristics of the barriers following a national standard for the evaluation of fish pass permeability (MAGRAMA, 2015). Free hydrological distance between two consecutive impermeable barriers was then measured. Refuge was estimated from an index according to different variables with importance on fish refuge. Substrate was characterized from a modified version of the substrate index in Ayllón (2009). Section, velocity and depth related measures were derived from cross-sectional profiles and the rest of physical habitat variables were obtained with data from an adapted version of River Habitat Survey (RHS; Environment Agency, 2003).

-Resources: Macroinvertebrate community and benthic organic matter data were derived from one benthic Surber sample (mesh 500 µm, 0.09 m2); each in 3 pools and 3 runs randomly selected along a 100 m reach. Surber samples were preserved in 97% ethanol. In the laboratory, samples were divided into < 1 mm and > 1 mm size fractions. Macroinvertebrate density was corrected for sample area in pools and runs and the sum of both was expressed as the total density of invertebrates. From the composite Surber samples, benthic organic matter was separated (wood, leaf litter, algae, seeds, FPOM and CPOM), dried at 70°C, weighed, ashed at 500°C for 4 h, and reweighed to yield ash

free dry mass (g/m^2). Benthic chlorophyll *a* and ephilitc carbon were obtained from six cobbles randomly collected (3 runs and 3 pools). Cobbles were brushed and from the composite slurry, two aliquots were filtered through separate pre-ashed 45-µm glass-fiber filters. Filters were transported cold (4°C) to the laboratory and preserved at -20°C until analysis. Chlorophyll *a* was extracted from one filter in 90% acetone at 4°C for 24h in the dark. Absorbance was read on a Hach-Lange DR-5000 UV/visible spectrophotometer and converted to pigment concentration. Epilithic biomass content was determined from a second filter using a modified version of the technique described by Sinsabaugh et al. (1991). Filters were dried at 95°C, weighed, ashed at 550°C for 2 h, and reweighed to yield ash free dry mass. Both measures were corrected for cobble surface area following Graham et al. (1988). Light was measured with HOBO Pendant® Temperature/Light 64K (Onset Computer Corporation, Cape Cod, MA, USA) loggers on the river bank facing up and Gross Primary Production (GPP) and Ecosystem Respiration (ER) were obtained using the single station open channel method based on dissolved oxygen dial changes. GPP and ER have been calculated in previous studies (see Rodríguez-Castillo, 2017; Rodríguez-Castillo et al., 2018).

For more detailed descriptive statistics (mean, standard deviation, minimum and maximum) of the environmental variables, see Supplementary material 4B Table S4B.1. Some environmental reach variables were available for all field sites with fish data (n = 76), but some were only available for a smaller number of river reaches (see Nº Field sites column in Supplementary material 4B Table S4B.1).

4.2.2 Data analysis

The data analysis in this study follows three consecutive steps. First, we selected a subset of independent environmental variables with potential influence on brown trout density by means of a correlation analysis. Second, we used Generalized Linear Models to analyse the relation between the environmental variables and brown trout density. Lastly, we used a Mantel test and partial Mantel test to identify the main factors determining the spatial patterns of fish density. All these different steps are described below in more detail. In all analysis, the number of cases was limited to the minimum number of sites with data available for all variables involved.

4.2.2.1 Selection of environmental variables with influence on fish density

We follow a two-step procedure to select the environmental variables with influence on the density of each age class. First, we calculated a correlation matrix (Spearman rank correlation) with the densities of each age-class and the values of the environmental variables. Only environmental variables with a correlation $\geq \left| 0.3 \right|$ and with an asymptotic significance *p-value* ($p < 0.05$) were selected for the following step. In the case of young-of-the-year (0+) density, we have included also as environmental variable the density of adults (2+) as proxy for a potential stock-recruitment relation (Touzeau & Gouzé, 1998). Second, to avoid potential problems with multicollinearity among the potential environmental variables, we calculated a second correlation matrix (Spearman rank correlation) between the environmental variables that were selected in the previous step for each age-class. When pairs of variables had a correlation $\geq \left| 0.7 \right|$ and an asymptotic significance *p-value* ($p < 0.05$), only one was retained. In these cases, the variable with the higher correlation coefficient with brown trout density was selected.

Analysis of correlation were developed using HMISC package version 4.0-3 (Harrell Jr, 2017) in the statistical software R 3.4.3 software (R Core Team, 2017) and the editor RStudio 1.0.143 (RStudio, 2015).

4.2.2.2 Relation between environmental variables and age-class density

A Generalized Linear Model (GLM; McCullagh & Nelder, 1989) with normal distribution was used to estimate the relation between the environmental variables selected in the previous step and the fish densities for the different brown trout age classes (0+, 1+ and 2+) as the dependent variable. Dependent variables were $\log_{10}$ transformed according to the procedure for logarithmic transformation in presence of zero data present in McCune and Grace (2002). The combination of environmental variables were selected based on the fitted model with smallest Akaike Information Criterion (AIC; Akaike, 1973) using a stepwise selection procedure in both directions (backward and forward). A GLM was fitted for each of the three considered brown trout age-classes and the percentage of deviance accounted for the model was evaluated. Plots of residuals from the final model against fitted values, normal probability plots, scale location plots and Cook`s distance plots were used to ensure linearity,

homoscedasticity and normality of the residuals. Finally, independence of residuals was checked with the Global Moran's I statistic and Euclidean distance.

GLM models were fitted using the statistical software R 3.4.3 software (R Core Team, 2017) and the editor RStudio 1.0.143 (RStudio, 2015). MASS package version 7.3-47 (Venables & Ripley, 2002) was used in the performance selection and modEVA package version 1.3.2 (Barbosa A.M. et al., 2016) was used to extract the variance. Global Moran's I statistic was developed in ArcMap 10.2 (ESRI, 2014).

4.2.2.3 Mantel test

Mantel test (Mantel, 1967) and partial Mantel test (Smouse et al., 1986) with the Spearman correlation coefficient were used to look for the best factors explaining spatial patterns of age-class density. The Mantel test estimates the correlation between two matrices whereas the partial Mantel test estimates the correlation between two matrices while controlling for the effects of a third matrix. These analyses have been widely used in population genetic studies to test for the effects of habitat fragmentation and isolation-by-distance. (e.g. Stelkens et al., 2012). We constructed a set of nine initial matrices. Three matrix corresponded to the Zero-adjusted Bray Curtis dissimilarity (Clarke et al., 2006) for each age-class fish density (0+, 1+ and 2+). We decided to use Zero-adjusted Bray Curtis dissimilarity to be able to use field sites with absence of fish. Two matrices corresponded to the hydrological distance matrix and Euclidean distance matrix between the UTM coordinates of every pair of field sites in meters. Another matrix was related to the presence of impermeable longitudinal barriers between pair of field sites (see Supplementary material 4A for permeable and impermeable barriers classification). It is a binary-coded matrix compiles of zero (absence of impermeable barriers) and ones (presence of impermeable barriers). The last three matrices included an environmental dissimilarity matrix for each age-class with the variables obtained in the second step of the selection of environmental variables with influence on fish densities. Environmental matrix was centered and scaled because of the different units of the variables and the dissimilarity matrix was obtained with the Euclidean distance method. Four simple Mantel test were performed for each age-class to test significant correlation between fish density and hydrological distance, Euclidean distance, presence of impermeable barriers and environmental variables. Afterwards, partial Mantel tests of significant variables were estimated.

All test were performed using the vegan package version 2.4-6 (Oksanen et al., 2008) in the statistical software R 3.4.3 software (R Core Team, 2017) and the editor RStudio 1.0.143 (RStudio, 2015). At each test, 999 permutations were performed. Zero-adjusted Bray-Curtis dissimilarity were obtained using the bray0 function in the ecole package v0.5-2017 (First Last, 2017). The environmental dissimilarity matrices were obtained using the vegan package. Hydrological distance matrix was obtained from the distance matrix created using the SSN package version 1.1.12 (Ver Hoef et al., 2014) and STARS toolbox version 2.0.3 (E. E. Peterson & Ver Hoef, 2014). Euclidean distance matrix and the matrix related to impermeable barriers were created using ArcMap 10.2 (ESRI, 2014).

4.3 Results

4.3.1 Description of density spatial patterns

Brown trout densities at the 76 field sites ranged from 0 to 1 ind/m^2 (0+; mean = 0.08 ind/m^2), 0 to 0.324 ind/m^2 (1+; mean = 0.039 ind/m^2) and 0 to 0.063 ind/m^2 (2+; mean = 0.01 ind/m^2). Fish were totally absent in 17 field sites while in 38 field sites were present the three age-classes (see Figure 4.2). Two age-classes were present in 14 field sites (8 sites with 0+ and 1+, 3 sites with 0+ and 2+ and 3 sites with 1+ and 2+) and only one class was present in 7 field sites (1 sites with 0+ and 6 sites with 1+). Deva area presents higher densities of 0+ than Cares area, concentrating the highest densities in headwater sites. Downstream reaches haves the lowest 1+ densities while headwaters have higher values. Finally, the higher values of 2+ density are in the middle area of the study catchment.

4.3.2 Selection of environmental variables with influence on fish densities

In the first step of the selection, 12, 6 and 14 environmental variables were significantly correlated to the density of 0+, 1+ and 2+ age-class, respectively (see Table S4C.1 in Supplementary material 4C; environmental variables). In the second step adult brown trout density (2+), catchment area (AREA), mean annual temperature within the segment wings (LC_TEM), mean annual catchment potential evapotranspiration (MN_EP), leaf litter (LEAF) and elevation (ELE), were selected for young-of-the-year. All these variables were positively correlated to 0+ densities, excluding LEAF and ELE

which were negatively correlated. In the case of juvenile density, LEAF, mean bankfull width (BW_MEA), benthic chlorophyll *a* (Chla) and gross primary production (GPP) were all negatively correlated, while substrate index (I_SUS) was positively correlated. Finally, active channel width (ACW_M), area occupied by denuded areas in the catchment (MN_DEN), mean water velocity (V_MEA), LEAF, area occupied by broadleaf forest within a 200 m buffer along the surveyed river reach (BF_BLF) and mean annual precipitation in the catchment wings (LC_PRE) were the variables retained for adult densities. For this age class, ACW_M, MN_DEN and V_MEA were positively correlated with adult density, while LEAF, BF_BLF and LC_PRE were negatively correlated. Variables representing the three different spatial scales were selected for both adults and young-of-the-year, while only reach variables were selected for juvenile. Graphical visualization of correlations from the first and second steps are included in the Figure 4.3.

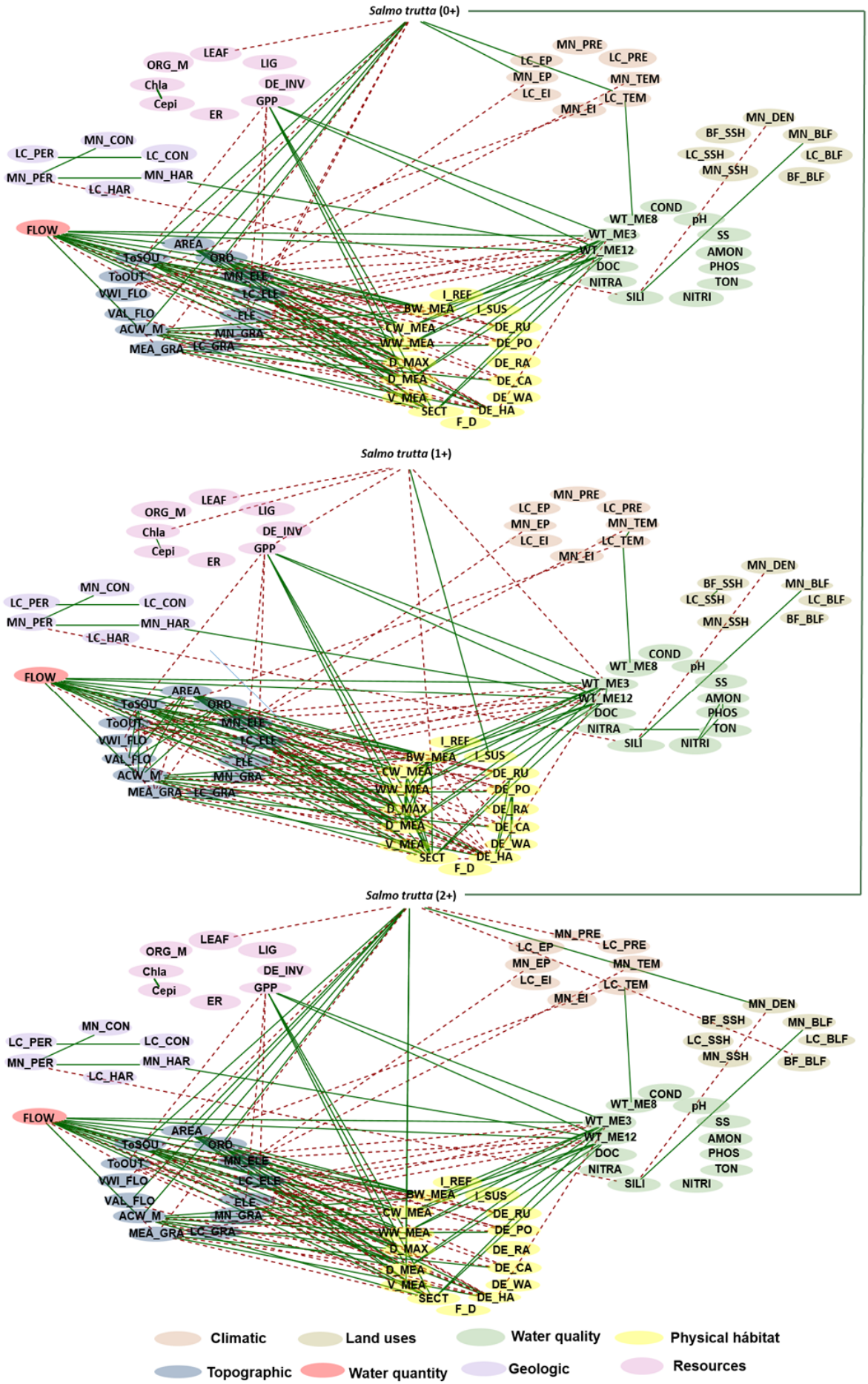
Salmo trutta (0+)
Salmo trutta (1+)
Salmo trutta (2+)
LEAF
ORG_M
LIG
Chla
DE_INV
Cepi
GPP
ER
MN_PRE
LC_EP
LC_PRE
MN_EP
MN_TEM
LC_EI
LC_TEM
MN_EI
MN_DEN
BF_SSH
MN_BLF
LC_SSH
LC_BLF
MN_SSH
BF_BLF
MN_CON
LC_PER
LC_CON
MN_PER
MN_HAR
LC_HAR
COND
WT_ME8
pH
FLOW
WT_ME3
SS
AREA
WT_ME12
AMON
ToSOU
ORD
DOC
PHOS
ToOUT
MN_ELE
NITRA
TON
VWI_FLO
LC_ELE
SILI
NITRI
I_REF
VAL_FLO
BW_MEA
I_SUS
ELE
CW_MEA
DE_RU
ACW_M
MN_GRA
WW_MEA
DE_PO
MEA_GRA
LC_GRA
D_MAX
DE_RA
D_MEA
DE_CA
V_MEA
DE_WA
SECT
DE_HA
F_D
Climatic
Land uses
Water quality
Physical hábitat
Topographic
Water quantity
Geologic
Resources

Figure 4.3. Graphical visualization of Spearman rank correlation results between environmental variables and three brown trout age classes surveyed in the Deva-Cares catchment. Groups of variables are presented with a color coding, while acronyms of the environmental variables are defined in the Table S4A.1 (Supplementary material 4A). Green lines represent significant positive correlations ($p < 0.05$), while dashed red lines represent significant negative correlations.

4.3.3 Relationships between environmental variables and fish densities: GLM models

The GLM model for young-of-the-year (0+) explained 25% of the deviance (adjusted $D^{2;}$ see Table 4.1) with 3 significant variables ($p < 0.05$) included, each corresponding to one of the spatial scale of study: adult brown trout density (2+), total catchment area (AREA) and mean annual temperature within the segment wings (LC_TEM). LC_TEM and 2+ positively influence the density of brown trout 0+, while AREA has negative influence. In the case of juveniles only two variables were selected explaining 30% of the deviance. Both reach variables, substrate index (I_SUS) and mean bankfull width (BW_MEA), showed a positive influence on the juvenile densities, but only BW_MEA was significant ($p < 0.05$). The deviance explained for the adult class was 24% with one catchment variable (area occupied by denuded areas [MN_DEN]), one segment variable (active channel width [ACW_M]) and two reach variables (area occupied by broadleaf forest within a 200 m buffer along the surveyed river reach [BF_BLF] and mean water velocity [V_MEA]). All variables were significant in the model except MN_DEN and all showed negative influence on brown trout 2+ except V_MEA.

Table 4.1. Generalized linear model summary for young-of-the-year (0+), juvenile (1+) and adult (2+), including predictor variables, parameter estimates, standard errors, *t-values*, *p-values*, D^2 and adjusted-D^2. Dependent variable was $\log_{10}$ transformed previously (see section 4.2.2.2 Relation of environmental variables and fish densities). Bold results are significant ($p <$ 0.05).

Age class	Covariates	b(SE)	*t-value*	Pr(≤|t|)	D^2	Adjusted D^2
0+	Intercept	-0.243 (0.272)	-0.894	0.377	0.323	0.248
	2+	8.687 (40.57)	2.141	**<0.05**		
	AREA	-0.001 (≈0.000)	-2.067	**<0.05**		
	LC_TEM	0.142 (0.041)	3.496	**<0.05**		

Table 4.1. (Continued)

Age class	Covariates	b(SE)	*t-value*	Pr(>|t|)	D^2	Adjusted D^2
1+	Intercept	0.018 (1.115)	0.015	0.987	0.359	0.301
	BW_MEA	-0.021 (0.009)	-2.489	**<0.05**		
	I_SUS	0.335 (0.227)	1.476	0.149		
2+	Intercept	1.177 (0.258)	4.562	**<0.05**	0.336	0.241
	ACW_M	-0.022 (0.008)	-2.723	**<0.05**		
	MN_DEN	-0.741 (0.390)	-1.898	0.066		
	V_MEA	2.119 (0.705)	3.006	**<0.05**		
	BF_BLF	-0.761 (0.282)	-2.695	**<0.05**		

4.3.4 Mantel test

Simple Mantel test revealed no significant correlation between brown trout densities and hydrological and Euclidean distance for adults and juveniles (see Table 4.2). There was however, a significant correlation between young-of-the-year and hydrological and Euclidean distance according to the simple Mantel test results. Environmental distance was positively correlated with the three age classes, increasing the value of the Mantel statistic r by decreasing the age class. The impermeable barriers matrix was also correlated with the three age classes, but in this case increasing the value of the Mantel statistic r by increasing the age class. Partial Mantel tests of significant variables for young-of-the-year showed that the influence of environmental distance is greater than the rest of the variables. Impermeable barriers and hydrological distance also presented significant values in the partial Mantel test controlling the effect of hydrological and Euclidean distance in the first case and impermeable barriers in the second case. For 1+ and 2+ Partial Mantel test of significant variables showed a significant effect of environmental distance when impermeable barriers was accounted for and vice versa (see Table 4.2).

Table 4.2. Simple Mantel and partial Mantel test between Zero-adjusted Bray Curtis dissimilarity for each age class (0+, 1+ and 2+), hydrological distance (HD), Euclidean distance (ED), presence of impermeable barriers (PERM_I) and dissimilarity environmental matrix (ENV). Bold results are significant ($p < 0.05$).

Mantel test		Mantel statistic r
0+	HD	**0.075**
	ED	**0.065**
	PERM_I	**0.078**
	ENV	**0.192**
1+	HD	0.010
	ED	0.015
	PERM_I	**0.082**
	ENV	**0.175**
2+	HD	0.008
	ED	0.002
	PERM_I	**0.105**
	ENV	**0.163**

Mantel test		Controlled by	Mantel statistic r
0+	HD	PERM_I	**0.049**
	HD	ENV	0.082
	HD	ED	0.039
	ED	PERM_I	0.042
	ED	ENV	-0.030
	ED	HD	0.015
	PERM_I	HD	**0.054**
	PERM_I	ED	**0.059**
	PERM_I	ENV	0.108
	ENV	HD	**0.156**
	ENV	ED	**0.17**
	ENV	PERM_I	**0.155**
1+	PERM_I	ENV	**0.082**
	ENV	PERM_I	**0.196**
2+	PERM_I	ENV	**0.137**
	ENV	PERM_I	**0.144**

4.4 Discussion

This study provides support for the hypothesis that environmental variables are playing and important role on determining brown trout density for each age-class, although the importance of environmental variables at different spatial scales differed among age-classes. Both environmental variables and connectivity are important on controlling the spatial variability of brown trout density, however, the importance of environmental variables increases, while the importance of connectivity decreases when moving from adults to young-of-the-year age-class. These results align with our hypothesis regarding dispersal-connectivity relationship, including the importance of connectivity for young-of-the-year, which was not contemplated in our initial expectations. Our hypothesis about the non-influence of the Euclidean distance is also supported by the results, while the hydrological distance only seems to be important for young-of-the-year density.

4.4.1 GLM adjustment

In general, the three GLM models presented low values of adjusted R^2 (see Table 4.1). One of the reasons for these low values could be because brown trout is a generalist species (Ayllón et al., 2010) and modelling densities of a generalist species usually produces worse adjustments than for specialist species (see González-Ferreras et al., 2016 for more information). This pattern might also emerge because of the total absence of individuals in many headwater reaches, in which the habitat is still suitable but it is inaccessible because of a lack of connectivity (see Figure 4.2). In order to test this, we performed the GLM analysis again but only considering locations where the species is present for each age-class (i.e., excluding zero data; see Supplementary material S4D for detailed results information). In this case, GLM models presented a much better adjustment to environmental factors (see Supplementary material S4D Table S4D.2). These results show that environmental variables alone are not able to explain fish density with presence and absence data expanding over the whole river network. That is, when considering locations where the brown trout is absent environmental predictors do not produce a high explanatory power of density patterns. However, taking into account only presence data (non-zero data), the explanatory power of the models increases substantially. Previous studies had shown higher values of adjusted R^2 using density data in reaches where trout is present (Alcaraz-Hernández et al., 2016) and better GLM fits with binomial data than density data for some fish species (e.g. plaice; Lauria et al., 2011).

4.4.2 The role of environmental variables on brown trout density

There were notable differences in the results obtained in the selection of environmental variables with correlation analysis and GLM among age classes, contributing to the idea that fish are spatially age-structured (Ayllón et al., 2013) and habitat requirements vary in relation to the developmental stage at consideration (Baglinière & Maisse, 1999). None of the variables selected in the three GLM were similar between them, showing that for each age class the relation of density and environmental variables is different including variables at reach, segment and catchment scale for 0+ and 2+ and only reach variables for 1+.

The most important predictor variable for 0+ densities was the density of adults. Previous studies have also shown a strong positive relationship between young-of-the-year densities and adult densities of the previous year in salmonids as brook trout (Huntsman & Petty, 2014). Both age classes (0+ and 2+) presented common variables correlated at different spatial scales (AREA, ACW_M, distance to the source [ToSOU], valley width index [VWI_FLO], order [ORD] and mean gradient [MEA_GRA]; see Table S4C.1). Most of these variables follow an increasing value from upstream to downstream and according to these results, density of 0+ is correlated positively with this pattern. This result contradicts the expected natural pattern where higher young-of-the-year densities are found in the upper part of the catchment (e.g. Maisse & Bagliniere, 1990; Baglinière & Maisse, 2002). This contrasting pattern in the Deva-Cares could be explained because young-of-the-year densities might have an important spatial dependence with the adult class, which tend to increase in densities in a downstream direction with increasing ecosystem size. In agreement to this explanation, Foldvik et al. (2010) found the maternal choice of breeding locations as a substantial driver of spatial heterogeneity in young-of-the-year densities of salmonid populations. Another variable that contributed to this 0+ density spatial pattern is LC_TEMP (air temperature), which follows an increasing gradient from upstream to downstream and from west (Cares axis) to east (Deva axis) where the highest densities of 0+ are found (see Figure 4.2). Different studies have reported that 0+ fish tend to be attracted to slightly warmer temperatures than other age-classes for feeding and growth (Sauter et al., 2001). However, our study cannot conclude any cause-effect relationship in this regard.

BW_MEA, a measure of stream size (Faustini et al., 2009), was the only significant variable of 1+ density in GLM models. The negative effect of BW_MEA on 1+ density indicates that the highest densities of 1+ are in the upstream parts of the catchment (Baglinière & Maisse, 1999). Rosenfeld et al. (2000) also observed a decline in juvenile cutthroat trout and coho salmon density with increasing stream size. In other studies, the juvenile age class has shown low mobility (Harcup et al., 1984). This could be one of the main causes why reach variables are more correlated with juvenile density (see Table S4C.1), since they might be more influenced by local habitat characteristics than connectivity. Although several studies have shown that presence of trout generated top-down cascading effects increasing benthic chlorophyll *a* (e.g. McIntosh & Townsend,

1996), in our study this environmental variable is negatively correlated to the density of 1+. This is because gross primary production (GPP) and benthic chlorophyll *a* (Chla) show a general pattern of downstream increases for the Deva-Cares catchment (Rodríguez-Castillo, 2017) and the lowest densities of 1+ (except for the total absence of the species) are in the downstream part of the catchment (see Figure 4.2). Water temperature in march (WT_ME3), which also increased downstream (Rodríguez-Castillo et al., 2018) was the unique water quality variable (negatively) correlated to juvenile density, which is important because aside from being the hatching month in our study area, it coincides with the beginning of the growing season (Parra et al., 2009), showing that physiological constrains might be important for this age class (Watz & Piccolo, 2011).

Adult brown trout density was mainly influenced by active channel width (ACW_M), highly correlated to pool size, and to water velocity (V_MEA). Water depth and velocity in combination with substrate or cover variables have been widely used in fish habitat suitability studies through habitat suitability curves (e.g. Heggenes & Saltveit, 1990; Ayllón et al., 2009) or in analysis of habitat selection in relation to energetic cost (Rosenfeld & Boss, 2001). Although these studies are conducted at the microhabitat scale and are not comparable to our data, these variables were also important for adult brown trout in our analysis (see Table S4C.1), increasing their value in the downstream direction (e.g. mean water depth and velocity).

4.4.3 Spatial patterns in the brown trout distribution: the role of environmental variables versus connectivity

The modification of the physical habitat or the alteration of connectivity in dendritic landscapes is of great importance for population dynamics of aquatic species (Fagan, 2002; Campbell Grant et al., 2007). In this study, the correlations between the environmental distances, impermeable barriers and the density of the three different age classes presented different strengths, evidencing changes on the importance of niche and dispersal principles on each age-class. Increasing mobility of the age class increased the importance of the dispersal-connectivity relation, while niche was more important in life stages with less mobility. We also performed again the Mantel and partial Mantel test with only river reaches with presence data (non-zero data) for each age-class (i.e. not considering absences; see Supplementary material 4D for a detailed results

information). In this case, only the environmental distances were important for all age-classes, showing that the absence of brown trout from the non-considered river reaches seems to be related to a lack of connectivity.

Our study showed that niche and dispersal were both important factors explaining the spatial variability of brown trout density at the river network scale. There are previous studies that define brown trout as metapopulations (Massa-Gallucci et al., 2010; Stelkens et al., 2014) and warn of the dangers of ignoring this structure for conservation (Cooper & Mangel, 1999). The absence of specific data (e.g. extinction and colonization processes) does not allow us to define whether brown trout in the Deva-Cares catchment constitutes or not a metapopulation. However, the existence of empty and occupied patches (i.e. differences on connectivity), and the effect that environmental variables might have on generating discrete and different quality patches in the Deva-Cares catchment are important conditions to support a metapopulation structure (sensu Schtickzelle & Quinn, 2007). Additional information from future specific studies such as genetic analysis or demographic data will improve our knowledge on the structure and spatial variability of brown trout populations in the Deva-Cares catchment and elsewhere. Moreover, this kind of information may allow us detect alterations in fish autoecological processes that were not considered in this study though caused by the loss of connectivity or habitat alteration, such as alteration of natural migration patterns (Carlos Garcia de Leaniz, 2008), reduction of genetic diversity and increased genetic differentiation (Wofford et al., 2005) or population persistence (Samia et al., 2015) among others.

4.5 Conclusions

In conclusion, this study evidences that different environmental variables are influencing brown trout density distribution at the river network scale and that the population is spatially age-structured. Environmental variables at the catchment, segment and reach scale are most important for young-of-the-year and adults, showing the young-of-the-year class a dependence on adult density, while the juvenile class is more influenced by river reach (local) variables. Impermeable barriers were also important on determining the spatial patterns of brown trout density. Such results indicate that the different age-classes are not only influenced by environmental factors, but that connectivity is playing an important role on structuring spatial patterns of

brown trout density in this river network. Connectivity mainly determines the presence/absence of the species, while the environmental variables influence more the carrying capacity (i.e. average density). Both factors should be considered together in order to better understand spatial patterns on trout densities, since increasing mobility increases the importance of the dispersal-connectivity relation and decreasing mobility increases the importance of the niche characteristics.

4.6 Supplementary material 4

4.6.1 Supplementary material 4A

Supplementary material 4A contains information about the environmental variables presented in the section "4.2.1.3 Environmental variables" of the Chapter IV. Some of the variables used have been derived from a wide range of digital resources in other previous studies. For brevity, we refer only to those works in which specific details are given on the methodological aspects used to derive them. For the rest of variables not used in previous studies, we present a detailed explanation of the methodology for obtain them. Variables whose methodology is similar have been grouped in this appendix for their methodology description (acronyms are defined in the Table S4A.1).

Table S4A.1. Initial set of environmental variables at different spatial scales.

TYPE	CODE	DEFINITION	UNITS	Scale
Topographic	AREA	Total catchment area	Km^2	Catchment
	ORD	Strahler order	1-7	Catchment
	MN_ELE	Mean catchment elevation from the considered river reach to the upper most river reach in the river network	m	Catchment
	LC_ELE	Mean catchment elevation within the segment wings	m	Segment
	ELE	Elevation of the river reach	m	Reach
	MN_GRA	Mean catchment gradient from the considered river reach to the upper most river reach in the river network	%	Catchment
	LC_GRA	Mean catchment gradient within the segment wings	%	Segment
	MEA_GRA	Mean gradient through the reach (vertical change/horizontal length)	-	Reach
	ACW_M	Active channel with	m	Segment
	VAL_FLO	Width of the valley floor at 2 x bankfull depth elevations above the channel	m	Segment
	VWI_FLO	Valley Width Index = VAL_FLOOR/ACW_M. Used as an indicator of channel confinement.	-	Segment
	ToOUT	Distance from river reach to river mouth	m	Segment
	ToSOU	Distance from river reach to river source	m	Segment

Table S4A.1. (Continued)

TYPE	CODE	DEFINITION	UNITS	Scale
Climatic	MN_TEM	Mean annual catchment temperature	ºC	Catchment
	LC_TEM	Mean annual temperature within the segment wings	ºC	Segment
	MN_PRE	Mean annual catchment precipitation	mm	Catchment
	LC_PRE	Mean annual catchment within the segment wings	mm	Segment
	MN_EP	Mean annual catchment potential evapotranspiration	mm	Catchment
	LC_EP	Mean annual potential evapotranspiration within the segment wings	mm	Segment
	MN_EI	Mean annual catchment evapotranspiration	mm	Catchment
	LC_EI	Mean annual evapotranspiration within the segment wings	mm	Segment
Land uses	MN_BLF	Area occupied by broadleaf forest from the considered reach to the most upper catchment point in the catchment	Parts per unit (º/1)	Catchment
	LC_BLF	Area occupied by broadleaf forest within the segment wings	Parts per unit (º/1)	Segment
	BF_BLF	Area occupied by broadleaf forest within a 200 m buffer along the surveyed river reach	Parts per unit (º/1)	Reach
	MN_DEN	Area occupied by denuded areas from the considered reach to the most upper catchment point in the catchment	Parts per unit (º/1)	Catchment
	MN_SSH	Area occupied by moors, heathland, scrub and shrubs from the considered reach to the most upper catchment point in the catchment	Parts per unit (º/1)	Catchment
	LC_SSH	Area occupied moors, heathland, scrub and shrubs within the segment wings	Parts per unit (º/1)	Segment
	BF_SSH	Area occupied by moors, heathland, scrub and shrubs within a 200 m buffer along the surveyed river reach	Parts per unit (º/1)	Reach
Geologic	MN_HAR	Average rock hardness from the considered reach to the most upper catchment point in the catchment	1-5	Catchment
	LC_HAR	Average rock hardness within the segment wings	1-5	Segment
	MN_CON	Average rock conductivity from the considered reach to the most upper catchment point in the catchment	1-5	Catchment

Table S4A.1. (Continued)

TYPE	CODE	DEFINITION	UNITS	Scale
	LC_CON	Average rock conductivity within the segment wings	1-5	Segment
	MN_PER	Average rock permeability from the considered reach to the most upper catchment point in the catchment	1-5	Catchment
	LC_PER	Average rock permeability within the segment wings	1-5	Segment
Water quantity	FLOW	Total discharge	m^3/s	Reach
Water quality	COND	Electric conductivity	µS/cm	Reach
	pH	pH	0-14	Reach
	SS	Total suspend solids	mg/L	Reach
	AMON	Ammonium	µgN/ L	Reach
	PHOS	Phosphate	µgP/ L	Reach
	TON	Total organic nitrogen	µgN/ L	Reach
	NITRI	Nitrite	µgN/ L	Reach
	SILI	Silicate	$\mu gSiO_2$/ L	Reach
	NITRA	Nitrate	µgN/ L	Reach
	DOC	Dissolved organic carbon	µgC/ L	Reach
	WT_ME12	Mean daily water temperature in December	ºC	Reach
	WT_ME3	Mean daily water temperature in March	ºC	Reach
	WT_ME8	Mean daily water temperature in August	ºC	Reach
Physical habitat	F_D	Free hydrological distance between impermeable barriers	m	Reach
	I_REF	Refuge Index for fish	0-4	Reach
	I_SUS	Substrate Index	1-6	Reach
	DE_RU	Density of runs	Nº/m	Reach
	DE_PO	Density of pools	Nº/m	Reach
	DE_RA	Density of rapids	Nº/m	Reach
	DE_CA	Density of cascades	Nº/m	Reach
	DE_WA	Density of waterfalls	Nº/m	Reach
	DE_HA	Density of total mesohabitats	Nº/m	Reach
	SECT	Cross-sectional area	m^2	Reach
	V_MEA	Mean water velocity	m^2/s	Reach
	D_MEA	Mean water depth	m	Reach
	D_MAX	Maximum water depth	m	Reach
	WW_MEA	Mean water width	m	Reach
	CW_MEA	Mean channel width	m	Reach
	BW_MEA	Mean bankfull width	m	Reach

Table S4A.1. (Continued)

TYPE	CODE	DEFINITION	UNITS	Scale
Resources	DE_INV	Invertebrates density	Ind/m^2	Reach
	LEAF	Leaf litter	g/m^2	Reach
	ORG_M	Total organic matter	g/m^2	Reach
	LIG	Light availability	Lux	Reach
	GPP	Gross primary production	gO_2/m^2d	Reach
	ER	Ecosystem respiration	gO_2/m^2d	Reach
	Chla	Benthic chlorophyll a	mg/m^2	Reach
	Cepi	Benthic epilithic carbon biomass	mg/m^2	Reach

Topography

- AREA, ORD, MN_ELE, LC_ELE, ELE, MN_GRA, LC_GRA, MEA_GRA, ACW_M, VAL_FLO, VWI_FLO, ToOUT, ToSOU. All the topographical variables were derived from a 25-m DEM using the NetMap platform (Miller, 2002b; Benda et al., 2016; www.terrainworks.org).

Climatic

- MN_TEM, LC_TEM, MN_PRE, LC_PRE, MN_EP, LC_EP, MN_EI, LC_EI. All the climatic variables were derived from monthly averages (1980-2006) calculated in a 1-km grid by interpolation of data recorded in weather stations of the Spanish network. These data were originally developed to be implemented into the Integrated System for Rainfall-Runoff modelling (in Spanish SIMPA model; Estrela & Quintas, 1996) by the Centre for Hydrographic Studies (CEDEX, Ministry of Public works and Ministry of Agriculture and Environment, Spain) for the assessment of water resources in natural regime at a national level.

Land uses

- MN_BLF, LC_BLF, BF_BLF, MN_DEN, MN_SSH, LC_SSH, BF_SSH. All the land uses variables were derived from the Soil Occupancy Information System (SIOSE) developed by the National Geographic Institute (IGN) of the Spanish Government at scale 1:25000.

Geologic

- MN_HAR, LC_HAR, MN_CON, LC_CON, MN_PER, LC_PER. All the geological variables were derived from the litostatigraphic and permeability map at scale 1:200000 developed by the Spanish Geologic and Mine Institute of the Spanish Government (IGME, 2006).

A more detailed description about the **topographic, climatic, land uses and geological** variables can be found in the IH rivers database (http://ihrivers.ihcantabria.com/InfoDatos.aspx). In addition, more information about this four types of variables can be found in all the previous studies where have been used and explained (see Fernandez et al., 2012; Fernández et al., 2014; Peñas et al., 2014; Álvarez-Cabria et al., 2016; González-Ferreras et al., 2016; Álvarez-Cabria et al., 2017; González-Ferreras & Barquín, 2017; Peñas et al., 2018; Rodríguez-Castillo et al., 2018 for more information).

The previous variables were derived from available GIS layers, while all the remaining variables defined below were surveyed in the river reaches.

Water quantity

- Flow was obtained from one cross-section profile performed with a portable Acoustic Doppler Velocimeter (FlowTracker Handheld Acoustic Doppler Velocimeter, SonTek/YSI Inc., United States) using the mid-section discharge equation. This data was obtained in conjunction with the fishing samplings (low-flow conditions in 2014).

Water quality

- CON, Ph, SS, AMON, PHOS, TON, NITRI, SILI, NITRA, DOC. The physicochemical characteristic of water were estimated according to Standard Methods for the Examination of Water and Wastewater (APHA. et al., 1999) during the low-flow season in 2014. CON and pH was in situ measured using an YSI 556 Multi-Parameter Handheld Meter (YSI Inc., Yellow Springs, OH, USA). A water sample per site was taken to determine the rest of variables, preserved in 250 mL polyethylene containers on ice and transported to the laboratory after sampling. SS were calculated by filtering samples through pre-weighted standard glass-fiber filters (1 μm) and weighing the residue retained on the filter after dried at 105ºC. NO3, NO2, PO4 and SiO2 concentrations were

determined by continuous flow analysis and UV spectrophotometry detection (AA3 Autoanalyzer, SEAL Analytical GmbH, Norderstadt, Germany). NH4 was estimated using continuous flow analysis and molecular fluorescence spectrophotometric detection (AA3 Autoanalyzer, SEAL Analytical GmbH, Norderstadt, Germany). DOC concentration was determined using catalytic combustion and CO2 detection with a non-dispersive infrared detector (Shimadzu TOC-V CSH Analyzer). Finally, TON concentration was measured by catalytic combustion and nitrogen monoxide detection by chemiluminescence (Shimadzu TOC-V CSH + TNM-L Analyzer). Values below the detection limit of these methods were substituted with half of the detection limit.

- WT_ME12, WT_ME3, WT_ME8. Water temperature was recorded during one year (from low-flow season 2014 to low-flow season 2015) each 30 minutes with temperature loggers (HOBO® model UA-002 temperature/Light data logger, Onset Computer Corporation, Cape Cod, MA, USA or Keller Series 36XW).

Physical habitat

- F_D was measured as the hydrological distance for each field site between impermeable obstacles. Obstacles were considered as permeable or impermeable longitudinal barriers depending on their physical characteristics, following a national standard for the evaluation of fish pass permeability (MAGRAMA, 2015). Permeable longitudinal barriers are obstacles with a maximum height of jump not greater than 1m, minimum depth of the pool exceeding the height of jump at least by 25%, and maximum width crest not greater than 0.5 m. Any other obstacle was considered permeable, unless it had a non-functional fish pass. Moreover, we also considered the obstacles located in the *Salmo salar* (Linnaeus, 1758) zone as permeable considering that *Salmo trutta* can also pass these obstacles. Based on these conditions, 57 out of the 139 longitudinal barriers comprised in the study area are impermeable. The barriers' locations are displayed in Figure 4.2 in the main text.

- I_REF. We designed the following refugee index to apply in our study area, in which each component receives points from 0 to 2 according to different variables with importance on fish refugee.

Component	Description	Value	Points
B1	Refugee due to the depth / transparency of the water column	Completely visible bed	0
		Partially visible bed	1
		Barely / not visible bed	2
B2a	Presence of ledges and caves (% length) on the right bank	Absence	0
		Presence (1-33%)	1
		Extensive (>33%)	2
B2b	Presence of ledges and caves (% length) on the left bank	Absence	0
		Presence (1-33%)	1
		Extensive (>33%)	2
B3	Presence of shade (% area)	Absence	0
		Presence (1-33%)	1
		Extensive (>33%)	2
B4	Presence of blocks > 1m diameter (% area)	Absence	0
		Presence (1-33%)	1
		Extensive (>33%)	2
B5	Presence of vegetation and roots (% area)	Absence	0
		Presence (1-33%)	1
		Extensive (>33%)	2
B6	Presence of wood > 20 cm diameter (% area)	Absence	0
		Presence (1-33%)	1
		Extensive (>33%)	2

The final value of the refuge index was calculated as I_REF = (B2a+B2B/2)+((B1+B3+B4+B5+B6)/5) and it varies from 0 to 4, from lowest to highest refuge availability.

- I_SUS. We used a modified version of the substrate index in Ayllón (2009) where the substrate index was calculated with field data from a modified version of River Habitat Survey (RHS; Environment Agency, 2003). We modified the RHS form in relation to length (100 m) and number of spot checks (5). RHS data can be obtained from an existing database with RHS data (www.rhs.ihcantabria.com). We used the channel substrate types dominant in each spot check of the section E of RHS and responds to the following formula:

  I_SUS = (6*BE+5*BO+4*CO+3*GP+2*SA+1*(EA+CL+SI))/Nº of spots

  Where I_SUS is the substrate index, BE is the number of spot checks where bedrock is dominant, BO is the number of spot checks where boulder is dominant, CO is the number of spot checks where cobble is dominant, GP is the number of spot checks where gravel/pebble is dominant, SA is the number of

spot checks where sand is dominant, EA+CL+SI is the number of spot checks where the sum of earth, clay and silt is dominant. Peat, artificial and not visible categories of RHS channel substrate were not considered because they were not registered in our study area. Substrate index is a number between 1 and 6, indicating an increasing order of substrate. This data was obtained in conjunction with the fishing samplings (low-flow conditions in 2014).

- DE_RU, DE_PO, DE_RA, DE_CA, DE_WA, DE_HA. We registered the mesohabitat sequence along 100 m of the river channel. Mesohabitat class used were rapid (RA), cascade (CA), waterfall (WA), run (RU), step (ST), glide (GL), dammed pool (DP), pool (PO), trench flow (TF), riffle (RI) and dry (DR). The density is determined by the total number of each mesohabitat class (or the total number for DE_HA) divided by the sampled length. This data was obtained in conjunction with the fishing samplings (low-flow conditions in 2014).

- SECT was defined by the stream cross–sectional channel area (m^2) computed from channel depth and width measures obtained from one cross-section profile performed with a portable Acoustic Doppler Velocimeter (FlowTracker Handheld Acoustic Doppler Velocimeter, SonTek/YSI Inc., United States). This data was obtained in conjunction with the fishing samplings (low-flow conditions in 2014).

- V_MEA. We measured the current velocity (0.6 depth from the water surface) in-situ from 5 cross-section profiles along 100 m of the field site with a portable flow meter Marsh-McBirney FLO-MATE model 2000 (in 4 cross-section profiles) and or a portable Acoustic Doppler Velocimeter (FlowTracker Handheld Acoustic Doppler Velocimeter, SonTek/YSI Inc., United States; in 1 cross-section profile). Moreover, in each cross-section we have taken this measure in 5 different distances (10%, 30%, 50%, 70% and 90% from the riverside). V_MEA is the average of the 5 cross-section profile.

- DE_MEA, D_MAX. We measured the water depth in-situ from 5 cross-section profiles along 100 m of the field site with a portable flow meter Marsh-McBirney FLO-MATE model 2000 (in 4 cross-section profiles) and or a portable Acoustic Doppler Velocimeter (FlowTracker Handheld Acoustic Doppler Velocimeter, SonTek/YSI Inc., United States; in 1 cross-section

profile). Moreover, in each cross-section we have taken this measure in 5 different distances (10%, 30%, 50%, 70% and 90% from the riverside). DE_MEA is the average of the 5 cross-section profile and D_MAX is the maximum value registered.

- WW_MEA, CW_MEA, BW_MEA were calculated with field data from a modified version of River Habitat Survey (RHS; Environment Agency, 2003) in relation to length (100 m) and number of spot checks (5). RHS data can be obtained from an existing database with RHS data (www.rhs.ihcantabria.com). In each spot check, we measured water width, channel width and bank full width according their definition in the RHS manual. WW_MEA, CW_MEA, BW_MEA measures are the average of the 5 spots checks. This data was obtained in conjunction with the fishing samplings (low-flow conditions in 2014).

Resources

- DE_INV was measured in the low flow season 2014. In each field site, we collected one benthic Surber sample (mesh 500 μm, 0.09 m^2), each in 3 pools and 3 runs randomly selected along a 100 m reach. In each reach, the total number of pools was counted, a unique number was assigned to each pool and 3 random numbers were drawn to select the sampled pools. The same procedure was repeated for the selection of the 3 runs. Surber samples were composited, preserved in 97% ethanol and returned to the laboratory. In the lab, samples were divided into < 1 mm and > 1 mm size fractions. Macroinvertebrate density was corrected for sample area (0.27 m^2) in pools and runs and the sum of both was expressed as the total density of invertebrates.

- LEAF, ORG_M. From the composite Surber samples, we separated wood, leaf litter, algae, seeds, FPOM and CPOM. Samples were dried to constant mass at 70°C, weighed, ashed at 500°C for 4 h, and reweighed to yield ash free dry mass (g/m*2*). Total organic matter was the sum of all categories, while leaf litter corresponds to the value of this category.

- LIG was measured at 5 min. intervals for a minimum of 72 hours during the low-flow season in 2014 using a HOBO Pendant® Temperature/Light 64K

(Onset Computer Corporation, Cape Cod, MA, USA) loggers on the river bank facing up.

- GPP, ER were estimated during the low-flow season in 2014 using the single-station open channel method based on dissolved oxygen diel changes. These values have been calculated in previous studies (Rodríguez-Castillo, 2017; Rodríguez-Castillo et al., 2018). Dissolved oxygen concentration and water temperature were measured with oxygen loggers (HOBO® model U26-001 dissolved oxygen data logger, Onset Computer Corporation, Cape Cod, MA, USA) calibrated in water-saturated air before deployment. Loggers were deployed in the stream bottom in well-mixed stream areas recording information at 5 minute intervals for a minimum of 72 hours. Net ecosystem production (NEP; gO_2.m-2.d-1) was estimated as the sum for a 24 hours period of the instant NEP. Instant NEP at time t was calculated as:

$$\mathrm{NEP(t)} = \mathrm{z} \times (\mathrm{dC/dt} - \mathrm{k} \times [\mathrm{Cs} - \mathrm{C}])$$

where C is the dissolved oxygen concentration measured, Cs the oxygen concentration at saturation, k is the reaeration coefficient and z is water depth. The reaeration coefficient for each stream was estimated using an empirical equation according to Melching and Flores (1999). Average night-time respiration (ANR; gO_2.m-2.h-1) was calculated as the average NEP during the night hours, while ER (gO_2.m-2.d-1) was estimated as the ANR extrapolated to 24 hours. GPP (gO_2.m-2.d-1) was calculated as $\mathrm{GPP} = \mathrm{NEP} - \mathrm{ER}$.

- Chla, Cepi. To estimate chlorophyll *a* concentration and epilithic carbon biomass of benthic biofilm, six cobbles were randomly collected from 3 runs and 3 pools. All cobbles were brushed individually into a tray and from the composite slurry, two aliquots of the same volume were filtered through three separate pre-ashed 45-µm glass-fiber filters. Filters were transported cold (4°C) to the laboratory and preserved at -20°C until analysis. Chlorophyll *a* was extracted from one filter in 90% acetone at 4°C for 24 h in the dark. Absorbance was read on a Hach-Lange DR-5000 UV/visible spectrophotometer and converted to pigment concentration. Epilithic biomass content was determined from a second filter using a modified version of the technique described by Sinsabaugh et al. (1991). Filters were dried to constant mass at 95°C, weighed,

ashed at 550°C for 2 h, and reweighed to yield ash free dry mass (g). Chlorophyll *a* concentration and epilithic biomass were corrected for cobble surface area, which was determined following Graham et al. (1988).

4.6.2 Supplementary material 4B

Supplementary material 4B contains the Table S4B.1 referred to in the main text of the Chapter IV.

Table S4B.1. Number of field sites with data and descriptive statistics (Mean, sd = Standard Deviation, min = Minimum and max = Maximum) of the fish data by age class and the initial set of environmental variables. Acronyms of the environmental variables are defined in Supplementary material Table S4A.1.

TYPE	CODE	Nº Field sites	mean	sd	min	max	Units
Fish Data	0+	76	0.080	0.151	0.000	1.000	Ind/m^2
	1+	76	0.039	0.061	0.000	0.324	Ind/m^2
	2+	76	0.010	0.016	0.000	0.063	Ind/m^2
Topographic	AREA	76	151.008	292.921	0.524	1186.757	Km^2
	ORD	76	3.684	1.560	1.000	7.000	1-7
	MN_ELE	76	1253.584	298.264	458.577	1996.228	m
	LC_ELE	76	569.577	393.704	10.200	1646.667	m
	ELE	76	563.171	391.793	10.000	1640.000	m
	MN_GRA	76	0.550	0.104	0.325	0.873	%
	LC_GRA	76	0.215	0.127	0.025	0.565	%
	MEA_GRA	76	0.059	0.059	0.000	0.380	-
	ACW_M	76	10.366	10.221	1.388	38.681	m
	VAL_FLO	76	59.488	66.505	22.700	418.300	m
	VWI_FLO	76	7.878	4.251	1.000	22.800	-
	ToOUT	76	40531.840	15116.770	552.926	60155.930	m
	ToSOU	76	14690.050	17102.140	550.631	64365.770	m
Climatic	MN_TEM	76	6.249	1.525	3.150	9.854	ºC
	LC_TEM	76	7.100	2.018	3.338	10.665	ºC
	MN_PRE	76	1106.579	116.858	942.314	1559.707	mm
	LC_PRE	76	1046.544	162.226	513.950	1501.837	mm
	MN_EP	76	709.559	55.162	597.312	870.226	mm
	LC_EP	76	753.196	87.227	602.768	915.382	mm
	MN_EI	76	442.138	37.806	386.729	563.768	mm
	LC_EI	76	457.825	90.720	268.387	725.654	mm

Table S4B.1. (Continued)

TYPE	CODE	Nº Field sites	mean	sd	min	max	Units
Land uses	MN_BLF	76	0.377	0.240	0.000	0.952	Parts per unit (º/1)
	LC_BLF	76	0.414	0.393	0.000	1.000	Parts per unit (º/1)
	BF_BLF	76	0.421	0.321	0.000	1.000	Parts per unit (º/1)
	MN_DEN	76	0.178	0.196	0.000	0.779	Parts per unit (º/1)
	MN_SSH	76	0.265	0.205	0.000	0.916	Parts per unit (º/1)
	LC_SSH	76	0.074	0.204	0.000	1.000	Parts per unit (º/1)
	BF_SSH	76	0.121	0.223	0.000	0.972	Parts per unit (º/1)
Geologic	MN_HAR	76	2.527	0.406	1.839	3.826	1-5
	LC_HAR	76	2.218	0.913	1.000	4.000	1-5
	MN_CON	76	2.667	0.746	1.139	4.477	1-5
	LC_CON	76	2.534	1.045	1.000	5.000	1-5
	MN_PER	76	2.332	0.870	1.000	4.269	1-5
	LC_PER	76	2.690	1.571	1.000	5.000	1-5
Water quantity	FLOW	47	0.702	1.202	0.002	3.825	m^3/s
Water quality	COND	41	235.278	57.395	77.400	350.000	µS/cm
	pH	41	8.399	0.387	7.680	8.960	0-14
	SS	41	38.965	14.656	8.600	67.600	mg/L
	AMON	41	20.720	17.316	7.000	90.000	µgN/ L
	PHOS	40	6.628	5.459	2.500	20.300	µgP/ L
	TON	41	333.032	159.639	31.515	749.311	µgN/ L
	NITRI	41	2.603	3.583	0.056	18.694	µgN/ L
	SILI	41	2971.943	1360.367	527.645	5563.616	µgSiO_2/ L
	NITRA	41	330.430	158.085	30.930	747.465	µgN/ L
	DOC	41	1.037	2.161	0.250	14.070	µgC/ L
	WT_ME12	37	7.221	2.067	1.824	10.371	ºC
	WT_ME3	37	7.076	1.903	2.241	9.540	ºC
	WT_ME8	37	14.450	2.897	7.843	18.789	ºC

Table S4B.1. (Continued)

TYPE	CODE	Nº Field sites	mean	sd	min	max	Units
Physical habitat	F_D	76	81657.100	83292.920	505.481	200950.200	m
	I_REF	44	2.393	0.990	0.000	3.400	0-4
	I_SUS	49	4.571	0.521	3.000	5.800	1-6
	DE_RU	54	0.055	0.037	0.000	0.130	Nº/m
	DE_PO	54	0.052	0.051	0.000	0.210	Nº/m
	DE_RA	54	0.016	0.016	0.000	0.060	Nº/m
	DE_CA	54	0.025	0.030	0.000	0.110	Nº/m
	DE_WA	54	0.012	0.020	0.000	0.090	Nº/m
	DE_HA	54	0.209	0.136	0.010	0.470	Nº/m
	SECT	46	2.213	3.203	0.039	13.998	m^2
	V_MEA	47	0.191	0.115	0.029	0.588	m^2/s
	D_MEA	47	0.408	0.290	0.118	1.548	m
	D_MAX	47	0.706	0.600	0.180	3.000	m
	WW_MEA	47	9.453	11.234	1.138	47.380	m
	CW_MEA	47	12.628	13.125	1.866	55.495	m
	BW_MEA	41	14.487	13.585	3.150	58.338	m
Resources	DE_INV	41	11797.560	8311.033	2148.148	39640.740	Ind/m^2
	LEAF	41	28.434	48.855	0.000	238.738	g/m^2
	ORG_M	41	531.194	2099.091	25.128	13486.980	g/m^2
	LIG	41	1300106.000	1411662.000	145827.452	6773619.000	Lux
	GPP	41	1.644	1.619	0.010	6.750	gO_2/m^2d
	ER	41	-2.337	1.597	-6.770	-0.190	gO_2/m^2d
	Chla	40	40.037	22.893	3.992	85.808	mg/m^2
	Cepi	40	7519.718	3509.381	2325.762	13607.470	mg/m^2

4.6.3 Supplementary material 4C

Supplementary material 4C contains the Table S4C.1 referred to in the main text of the Chapter IV.

Table S4C.1. Subset of environmental variables correlated with young-of-the-year (0+), juvenile (1+) and adults (2+) brown trout densities (Spearman rank correlation $\geq |0.3|$). Bold variables are uncorrelated environmental variables (Spearman rank correlation $< |0.7|$). Acronyms of the environmental variables are defined in the Table S4A.1.

Fish Variable	Environmental Variable	Correlation Coefficient	Correlated to:
0+	**2+**	**0.512**	
	AREA	**0.411**	ACW_M, MEA_GRA, ORD, ToSOU, VWI_FLO
	ACW_M	0.406	AREA, MEA_GRA, ORD, ToSOU, VWI_FLO
	ToSOU	0.405	ACW_M, AREA, MEA_GRA, ORD, VWI_FLO
	VWI_FLO	-0.402	AREA, ACW_M, ORD, ToSOU
	LEAF	**-0.399**	
	MEA_GRA	-0.396	ACW_M, AREA, ORD, ToSOU
	ORD	0.355	ACW_M, AREA, MEA_GRA, ToSOU, VWI_FLO
	ELE	**-0.310**	LC_ELE
	LC_ELE	-0.310	ELE
	LC_TEM	**0.309**	
	MN_EP	**0.307**	
1+	**LEAF**	**-0.450**	
	BW_MEA	**-0.369**	WT_ME3
	Chla	**-0.363**	
	GPP	**-0.357**	
	WT_ME3	-0.343	BW_MEA
	I_SUS	**0.315**	
2+	**ACW_M**	**0.577**	AREA, D_MEA, MEA_GRA, ORD, ToSOU, VAL_FLO, VWI_FLO, WW_MEA
	AREA	0.576	ACW_M, D_MEA, MEA_GRA, ORD, ToSOU, VAL_FLO, VWI_FLO, WW_MEA
	ToSOU	0.563	ACW_M, AREA , D_MEA, MEA_GRA, ORD, VAL_FLO, VWI_FLO, WW_MEA
	VWI_FLO	-0.554	ACW_M, AREA, ORD, ToSOU
	ORD	0.489	ACW_M, AREA , D_MEA, MEA_GRA, ToSOU, VAL_FLO, VWI_FLO, WW_MEA

Table S4C.1. (Continued)

Fish Variable	Environmental Variable	Correlation Coefficient	Correlated to:
	LEAF	**-0.489**	
	VAL_FLO	0.440	ACW_M, AREA, MEA_GRA, ORD, ToSOU
	MEA_GRA	-0.378	ACW_M, AREA , D_MEA, ORD, ToSOU, VAL_FLO, WW_MEA
	MN_DEN	**0.374**	
	V_MEA	**0.359**	
	BF_BLF	**-0.325**	
	D_MEA	0.321	ACW_M, AREA, MEA_GRA, ORD, ToSOU, WW_MEA
	LC_PRE	**-0.312**	
	WW_MEA	0.302	ACW_M, AREA, D_MEA, MEA_GRA, ORD, ToSOU

4.6.4 Supplementary material 4D

Supplementary material 4D contains the results of the analysis presented in the discussion of the main text of the Chapter IV. The methodology followed has been the same as that presented in the text excluding the field data where each-age class was absent.

Table S4D.1. Subset of environmental variables correlated with young-of-the-year (0+), juvenile (1+) and adults (2+) brown trout densities (Spearman rank correlation $\geq |0.3|$). Bold variables are uncorrelated environmental variables (Spearman rank correlation $< |0.7|$). Acronyms of the environmental variables are defined in the Table S4A.1.

Fish Variable	Environmental Variable	Correlation Coefficient	Correlated to:
0+	**WT_ME3**	**-0.567**	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MEA, WW_MEA, CW_MEA, BW_MEA, WT_ME12
	FLOW	-0.471	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, SECT, D_MEA, D_MAX, ,WW_MEA, CW_MEA, BW_MEA, WT_ME3, WT_ME12
	D_MAX	**-0.461**	AREA, ORD, ACW_M, ToSOU, FLOW, SECT, D_MEA, WW_MEA, CW_MEA, BW_MEA
	WT_ME12	-0.460	AREA, ORD, MEA_GRA, ACW_M, ToSOU, FLOW, SECT, D_MEA, WW_MEA, CW_MEA, BW_MEA, WT_ME3
	D_MEA	-0.458	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MAX, WW_MEA, CW_MEA, BW_MEA, WT_ME3, WT_ME12
	ACW_M	-0.407	AREA, ORD, MEA_GRA, VAL_FLO, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, WW_MEA, CW_MEA, BW_MEA, WT_ME3, WT_ME12
	BW_MEA	-0.406	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, WW_MEA, CW_MEA, WT_ME3, WT_ME12
	WW_MEA	-0.401	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, CW_MEA, BW_MEA, WT_ME3, WT_ME12
	CW_MEA	-0.400	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, WW_MEA, BW_MEA, WT_ME3, WT_ME12

Table S4D.1. (Continued)

Fish Variable	Environmental Variable	Correlation Coefficient	Correlated to:
	AREA	-0.395	ORD, MEA_GRA, ACW, VAL_FLO, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, WW_MEA, CW_MEA, BW_MEA, WT_ME3, WT_ME12
	ORD	-0.390	AREA, MEA_GRA, ACW, VAL_FLO, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, WW_MEA, , CW_MEA, BW_MEA, WT_ME3, WT_ME12
	ToSOU	-0.387	AREA, ORD, MEA_GRA, ACW_M, VAL_FLO, DE_HA, FLOW, SECT, D_MEA, D_MAX, WW_MEA, , CW_MEA, BW_MEA, WT_ME3, WT_ME12
	V_MEA	**-0.381**	
	MN_CON	**-0.371**	
	DE_HA	0.358	AREA, ORD, MEA_GRA, ACW_M, ToSOU, FLOW, SECT, D_MEA,
	VAL_FLO	-0.344	AREA, ORD, MEA_GRA, ACW_M, ToSOU, WW_MEA, , CW_MEA, BW_MEA, WT_ME3
	MEA_GRA	0.338	AREA, ORD, ACW_M, VAL_FLO, ToSOU, DE_HA, FLOW, SECT, D_MEA, WW_MEA,CW_MEA, BW_MEA, WT_ME3, WT_ME12
	SECT	-0.335	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, D_MEA, D_MAX, WW_MEA, CW_MEA, BW_MEA, WT_ME3, WT_ME12
1+	**F_D**	**-0.514**	
	WT_ME3	**-0.513**	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MEA, WW_MEA, CW_MEA, BW_MEA, GPP
	LEAF	**-0.508**	
	DE_RU	**0.487**	ORD, ToSOU, DE_HA, FLOW, SECT, WW_MEA, CW_MEA, BW_MEA
	WW_MEA	-0.485	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, DE_RU, DE_CA, FLOW, SECT, D_MEA, D_MAX, CW_MEA, BW_MEA, GPP, WT_ME3
	BW_MEA	-0.480	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, DE_RU, DE_CA, FLOW, SECT, D_MEA, D_MAX, CW_MEA, WW_MEA, WT_ME3
	I_SUS	**0.476**	
	DE_CA	**0.469**	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_PO, DE_HA, FLOW, SECT, WW_MEA, CW_MEA, BW_MEA
	CW_MEA	-0.456	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, DE_RU, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, WT_ME3

Table S4D.1. (Continued)

Fish Variable	Environmental Variable	Correlation Coefficient	Correlated to:
	FLOW	-0.453	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, DE_RU, DE_CA, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	SECT	-0.448	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, DE_RU, DE_CA, FLOW, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3, GPP
	DE_HA	0.442	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_RU, DE_CA, DE_PO, DE_WA, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	Cepi	**-0.430**	GPP, Chla
	ORD	-0.426	AREA, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_RU, DE_CA, DE_HA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	WT_ME8	**-0.420**	DE_WA
	D_MAX	**-0.420**	AREA, ORD, LC_ELE, ACW_M, ToSOU, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA
	GPP	-0.408	LC_ELE, ToSOU, SECT, D_MEA, WW_MEA, ER, WT_ME3
	ToSOU	-0.398	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, DE_RU, DE_CA, DE_HA, DE_PO, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3, GPP
	ER	**0.384**	GPP
	MEA_GRA	0.383	AREA, ORD, ACW_M, ToSOU, DE_CA, DE_HA, DE_PO, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	D_MEA	-0.382	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3, GPP
	AREA	-0.370	ORD, LC_ELE, MEA_GRA, ACW_M, ToSOU, DE_CA, DE_HA, DE_PO, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	Chla	-0.364	Cepi
	ACW_M	-0.360	AREA, ORD, LC_ELE, MEA_GRA, ToSOU, DE_CA, DE_HA, DE_PO, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	DE_PO	0.359	AREA, MEA_GRA, ACW_M, ToSOU, DE_CA, DE_WA, DE_HA
	DE_WA	0.319	DE_PO, DE_HA, WT_ME8

Table S4D.1. (Continued)

Fish Variable	Environmental Variable	Correlation Coefficient	Correlated to:
	LC_ELE	0.302	AREA, ORD, ACW_M, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3, GPP
2+	**WT_ME3**	**0.575**	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, GPP, WT_ME12
	WT_ME8	**0.549**	LC_TEM,
	F_D	**0.516**	BW_MEA
	ToOUT	0.494	AREA, LC_ELE, ELE, ACW_M, ToSOU, LC_TEM, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA, GPP, WT_ME12, WT_ME3
	WW_MEA	0.491	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, CW_MEA, WT_ME12, WT_ME3
	D_MAX	0.489	AREA, ORD, LC_ELE, ELE, ACW_M, ToSOU, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	WT_ME12	0.480	AREA, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, LC_PRE, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA, WT_ME3, GPP
	SECT	0.468	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, DE_CA, FLOW, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3
	LC_ELE	0.465	AREA, ORD, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3, GPP
	LC_PRE	**0.463**	WT_ME12
	ELE	0.454	AREA, ORD, LC_ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3, GPP
	FLOW	0.445	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, DE_CA, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3
	CW_MEA	0.439	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3

Table S4D.1. (Continued)

Fish Variable	Environmental Variable	Correlation Coefficient	Correlated to:
	D_MEA	0.427	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, FLOW, SECT, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3
	ACW_M	0.414	AREA, ORD, LC_ELE, ELE, MEA_GRA, ToOUT, ToSOU, DE_HA, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3
	BW_MEA	0.410	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3, F_D
	MEA_GRA	0.407	AREA, ORD, LC_ELE, ELE, ACW_M, ToSOU, DE_HA, DE_CA, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3
	ToSOU	0.406	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, DE_HA, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3
	ORD	0.404	AREA, LC_ELE, ELE, MEA_GRA, ACW_M, ToSOU, DE_HA, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	AREA	0.398	ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToOUT, ToSOU, DE_HA, DE_CA, FLOW, SECT, D_MEA, D_MAX, BW_MEA, WW_MEA, CW_MEA, WT_ME12, WT_ME3
	GPP	0.394	LC_ELE, ELE, ToOUT, WT_ME12, WT_ME3
	LC_TEM	0.392	ToOUT, LC_TEM,
	LEAF	**0.381**	
	Chla	**0.365**	
	DE_HA	0.362	AREA, ORD, LC_ELE, ELE, MEA_GRA, ACW_M, ToSOU, DE_CA, FLOW, SECT, D_MEA, BW_MEA, WW_MEA, CW_MEA, WT_ME3
	DE_CA	**0.354**	AREA, ORD, MEA_GRA, ACW_M, ToSOU, DE_HA, FLOW, SECT, BW_MEA, WW_MEA, CW_MEA
	DE_RA	**0.352**	
	MN_GRA	**0.351**	
	LC_BLF	**0.314**	
	LC_EI	**0.310**	

Table S4D.2. Generalized linear model summary for young-of-the-year (0+), juvenile (1+) and adult (2+), including predictor variables, parameter estimates, standard errors, *t-values*, *p-values*, D^2 and adjusted-D^2. Dependent variable was log_{10} transformed previously (see section 4.2.2.2 Relation between environmental variables and age-class density). Bold results are significant ($p < 0.05$).

Age class	Covariates	b(SE)	*t-value*	Pr(>|t|)	D^2	Adjusted D^2
0+	Intercept	1.698 (0.228)	7.438	**<0.05**	0.393	0.291
	V_MEA	-1.141 (0.544)	-2.098	**<0.05**		
	MN_CON	-0.140 (0.080)	-1.737	0.094		
	VAL_FLO	-0.001 (0.001)	-2.098	**<0.05**		
1+	Intercept	-0.095 (0.764)	-0.124	0.902	0. 616	0.566
	F_D	-0.000004 (≈0.000)	-4.600	**<0.05**		
	I_SUS	0.409 (0.162)	2.522	0.149		
2+	Intercept	0.848 (0.382)	2.219	**<0.05**	0.708	0.616
	WT_ME3	0.092 (0.043)	2.118	**<0.05**		
	F_D	-0.000002 (≈0.000)	-2.313	**<0.05**		
	LC_PRE	-0.001 (≈0.000	-2.069	0.052		
	DE_CA	8.388 (2.823)	2.972	**<0.05**		
	DE_RA	13.01	3.827	**<0.05**		

Table S4D.3. Simple Mantel and partial Mantel test between Bray Curtis dissimilarity for each age class (0+, 1+ and 2+), hydrological distance (HD), Euclidean distance (ED), presence of impermeable barriers (PERM_I) and dissimilarity environmental matrix (ENV). Bold results are significant ($p < 0.05$) and italic results are marginally significant ($p < 0.1$).

Mantel test		Mantel statistic r
0+	HD	0.039
	ED	0.050
	PERM_I	0.047
	ENV	*0.136*
1+	HD	-0.026
	ED	**0.121**
	PERM_I	0.004
	ENV	**0.289**
2+	HD	0.049
	ED	**0.188**
	PERM_I	0.066
	ENV	**0.240**

Mantel test		Controlled by	Mantel statistic r
1+	ED	ENV	0.064
	ENV	ED	**0.218**
2+	ED	ENV	**0.170**
	ENV	ED	**0.160**

Chapter V

Effects of altered river network connectivity in the distribution of *Salmo trutta*: insights from a metapopulation model

Chapter V: Effects of altered river network connectivity in the distribution of *Salmo trutta*: insights from a metapopulation model.

This study, performed by González-Ferreras, A.M, Bertuzzo E., Barquín, J, Carraro, L., Alonso, C. and Rinaldo, A., has been submitted for publication in the journal Freshwater Biology and is under review.

Abstract

Network connectivity is a key feature of rivers that affects patterns and processes in lotic ecosystems. Few studies have considered how changes in river reach connectivity might affect ecosystem attributes at a whole river network scale. The use of population dynamics models of keystone species at a river network scale is crucial to explore how the effects of altered natural connectivity patterns might propagate through a river network. In this study, we present a metapopulation model to estimate the spatial distribution of the population density of brown trout (*Salmo trutta*), an ecologically and socioeconomically important top-predator. The model accounts for the presence of barriers that limit longitudinal connectivity in upstream and downstream directions. The model estimates the spatial distribution of densities of three age-classes (young-of-the-year, juveniles and adults) in all river reaches that make up the network based on topology, connectivity and population dynamics (e.g. age-class specific mortality, spawning, age-class dispersal and spawning migration patterns). 75% of the modelled results fell within the 95% confidence intervals of the empirical data (84.6% for young-of-the-year, 69.2% for juveniles and 69.2% for adults). The removal of all longitudinal barriers to migration in the river network (re-naturalization of the whole catchment) produced an increase in brown trout density in the most downstream reaches of the river network and lowered fish densities in the upstream portion of the network when bias in juvenile and adult movement direction was assumed. Furthermore, the removal of a single obstacle affected fish density even in distant tributaries. The proposed model is an appropriate tool for the evaluation of spatial patterns of brown trout density at a river network scale and for the assessment of the impact of altered connectivity. This might help simulate the results of management strategies regarding river connectivity and

showing where population decreases or increases could be expected, although empirical knowledge of overall trout movement in the studied river networks is required for drawing realistic scenarios.

5.1 Introduction

Dendritic geometry is common to river systems, being connectivity a function of network topology (Campbell Grant et al., 2007). Connectivity is considered one of the fundamental properties of rivers (Moore, 2015), and it influences different patterns and processes in lotic ecosystems (Fagan, 2002; Benda et al., 2004; Bertuzzo et al., 2007; Muneepeerakul et al., 2007; Muneepeerakul et al., 2008; Rodriguez-Iturbe et al., 2009; Carrara et al., 2012). From a hydrological perspective, connectivity is defined as "water-mediated transfer of matter, energy, or organisms within or between elements of the hydrological cycle" (Pringle, 2001). Although river networks are naturally fragmented (e.g. low flows or waterfalls), human actions have further disconnected these habitats (Fuller et al., 2015), changed connections within and between ecosystems over a wide range of habitat types (Crook et al., 2015) and altered the nature of hydrologic connectivity on local, regional and global spatial scales (Pringle et al., 2000; D. M. Rosenberg et al., 2000). One of the most important human impacts in this regard is the presence of longitudinal barriers, which may be physical (e.g. dams), biological (e.g. diseases) or physicochemical (e.g. stream temperature or pollution plumes). For example, by the end of the 20th century there existed more than 45,000 large dams in over 140 countries (WCD, 2000) and the number of small dams was (and still is) considerably larger (in the United Stated alone there are more than 2 million; Smith et al., 2002).

Dams provide many benefits to society for drinking water, irrigation, hydropower or recreation (WCD, 2000). However, dams and weirs also have adverse effects on aquatic organisms by reducing upstream and downstream migration and changing water quality and/or habitat conditions (Poff & Hart, 2002). One of the biological groups that are most threatened by the loss of connectivity is migratory fish (Sheer & Steel, 2006), especially migratory fish covering long distances. Nonetheless, other groups have also been affected by the presence of barriers such as mammals (Pavanato et al., 2016), macroinvertebrates (Cortes et al., 1998), plants (Jansson et al., 2000) and crustaceans (Miya & Hamano, 1988) among others. Site-specific and local impacts of dams have

been extensively investigated (e.g. Lessard & Hayes, 2003; Greathouse et al., 2006; Gardner et al., 2013). However, small scale studies might prove insufficient for understanding ecosystems repercussions at a catchment scale (Campbell Grant et al., 2007). Up to date, few studies have addressed how changes to river reach connectivity might affect ecosystem attributes at a river network scale (but see Ziv et al., 2012), for example, by determining the spatial extent of the effect of such alteration throughout the entire river network (e. g. Yeakel et al., 2014). Some studies have quantified the global longitudinal connectivity loss in riverine systems as well as the relative contribution of every barrier (Erős et al., 2011; Segurado et al., 2013; G. Rincón et al., 2017). These studies are often based on graph theory and measures of connectivity to determine potential areas for conservation. However, the biological information incorporated in these studies is rather limited (e.g. mortality rates, survival rates) and, therefore, it is very difficult to understand how different population traits interact with river connectivity to generate the observed spatial patterns. In this regard, building population dynamics models of keystone river species at a river network scale is a promising approach to better understand how the effects of altered connectivity might propagate through a river network (Charles et al., 2000). Keystone species (Paine, 1966) has a strong impact on ecosystem processes and biological communities through predation, competition or ecosystem engineering (see Cottee-Jones & Whittaker, 2012 for keystone concepts). Several species linked to freshwater ecosystems have been identified as keystone species in different areas such as *Castor fiber* (Linnaeus, 1758) (Janiszewski et al., 2014), *Chen caerulescens caerulescens* (Linnaeus, 1758) (Kerbes et al., 1990) or anadromous fishes (Willson & Halupka, 1995). In this regard, salmonids are often considered as keystone species in river ecosystems (e.g. brown and brook trout; Tzilkowski, 2005). Moreover, salmonid populations have been considered as dendritic metapopulations (Dunham & Rieman, 1999; Consuegra & García de Leániz, 2006), and as such, river network connectivity has a major influence on their population dynamics and genetics (Labonne et al., 2008).

Brown trout (*Salmo trutta* Linnaeus, 1758) is one of the most widespread salmonid fish species in European inland waters and its natural distribution is fundamentally restricted to this area (Elliott, 1989c), although it has been introduced to many countries worldwide (Elliott, 1989c). Moreover, brown trout is of great importance from an ecological and socio-economic point of view, as it is the top-predator fish in many river

systems (Jensen et al., 2008; Sánchez-Hernández, 2016) and one of the most popular and important sport-fishing species (Almodovar & Nicola, 1998). The ecology of brown trout has been studied for many years (Elliott, 1994; B. Jonsson & Jonsson, 2011) and several studies on population dynamics have been conducted in different regions (Mortensen, 1977; Bagliniére et al., 1989; Elliott, 1994; Lobón-Cerviá, 2012). However, there is still much uncertainty regarding knowledge of how different age-classes of brown trout move within and among river networks and how these movements determine current spatial density patterns in river networks. Young-of-the-year salmonids tend not to move much at the river reach scale (Steingrímsson & Grant, 2003), while juvenile salmonids have been reported to move mainly upstream (N. P. Peterson, 1982; Kahler et al., 2001), while adults tend to move upstream for spawning (Banks, 1969), but they might also move downstream to look for more suitable habitats as they grow and became larger (Sanz et al., 2011). As a result of this complexity in movement behavior among life stages, studies that model brown trout population dynamics at the scale of a whole real river network considering the actual spatial structure of the network, longitudinal connectivity and trout movement are scarce (Frank et al., 2011).

The present study aims at modeling the average spatial patterns of density of brown trout and exploring the effects of connectivity and dispersal on the average spatial patterns of brown trout density in the Deva-Cares catchment in Northern Spain. A metapopulation model of brown trout demography is developed and applied to the Deva-Cares catchment, which is affected by the presence of numerous longitudinal barriers. The model, calibrated using field data on fish population density, is used to explore scenarios of river connectivity, where either one or all longitudinal barriers are removed (to evaluate local or global effects of connectivity). We hypothesize that changes in connectivity will impact differently the average density of the considered age-classes, owing to their different propensity to movement. Finally, we also look at the effect of different brown trout movement biases on the average spatial densities of the different age-classes.

5.2 Methods

5.2.1 River network

The river network (Figure 5.1) covers the perennial reaches of the Deva-Cares catchment in low-flow conditions (for more information see González-Ferreras & Barquín, 2017 and Chapter III). Temporary reaches were assumed not to be permanently inhabited by brown trout, and were not considered in the study. The river network was divided into 623 river reaches (hereafter termed nodes - mean length 1000 m) delimited by sources and confluences. The study area included 139 longitudinal barriers (see Figure 5.1) of which 102 are of anthropogenic origin and 37 are natural.

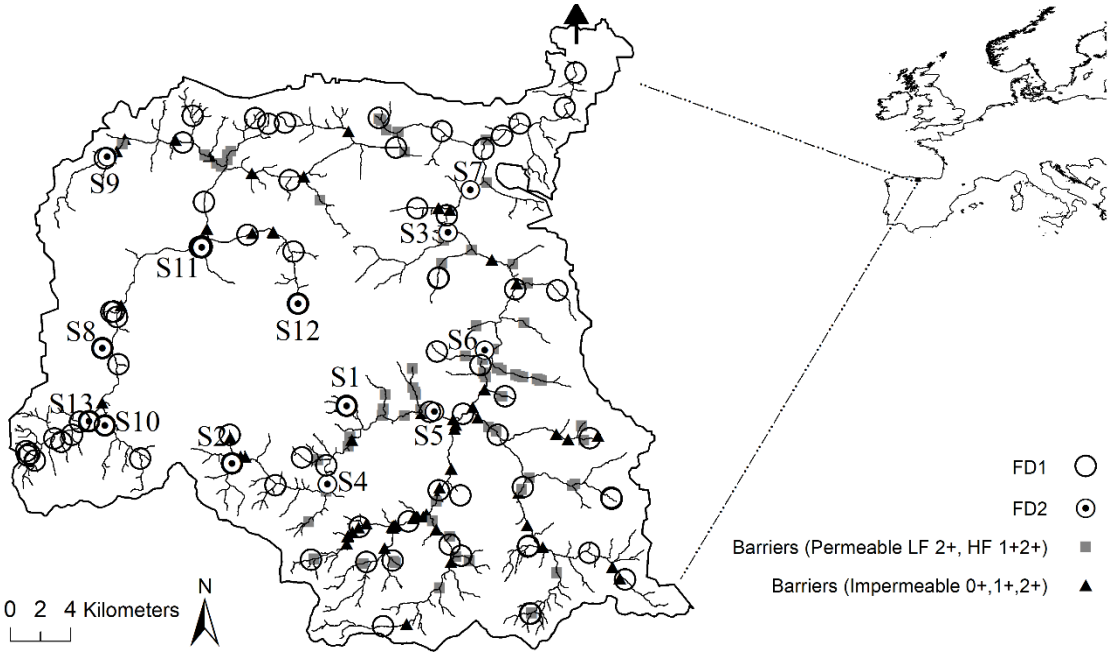

Figure 5.1. Map of the study area and representation of the Deva-Cares river network. Field sites from the two different data sets (FD1 and FD2) used in the study, longitudinal barriers (high flow-HF and low flow-LF) and permeability for the three age-classes (0+, 1+ and 2+) are also located.

5.2.2 Fish surveys

Two data sets (FD1 and FD2) were employed in our study with different purposes. Both data sets were obtained from field surveys using electrofishing techniques. Each field survey was carried out on a minimum area that was representative of the river reach. A portable electric fishing device (power 1.3 kW) was used, with direct current generation

of 300 to 500 V, or pulsating up to 940 V with variable frequency (25 to 100 Hz). The successive catch method was performed and the Carle & Strub method (Carle & Strub, 1978) was used for abundance population estimates.

Data set FD1 contains information on presence-absence of brown trout in 76 sites (see Figure 5.1) of the Deva-Cares river network, representing the spatial variability of the species. Field surveys were carried out in August, September and October (low flow conditions) in 2014. These data were used to establish the initial brown trout presence-absence in each node of the network. Data set FD2 gathers data from 13 sites sampled at least three times (once a year) between 2010 and 2015. Field surveys were carried out in August, September or October over multiple years (between 2010 and 2015) to represent temporal variability in fish densities. Captured brown trout individuals were also classified into three age-classes (young-of-the-year (0+), juvenile (1+) and adult (2+)) by means of a visual analysis based on the frequency distribution of the fork length (Petersen, 1986). This data set was used to estimate fish carrying capacity and for model calibration, as further explained below.

5.2.3 Metapopulation model

A metapopulation is defined as a "set of local populations within some larger area, where typically migration from one local population to at least some other patches is possible" (*sensu* Hanski & Simberloff, 1997). Our metapopulation model includes a freshwater brown trout population subdivided into three age-classes: young-of-the-year (0+), juveniles (1+) and adults (2+). The model is formulated under a spatially explicit, time-hybrid continuous-discrete framework (see Figure 5.2a), in analogy with the epidemiological model for proliferative kidney disease in salmonids of Carraro et al. (2016; 2018). A set of ordinary differential equations expresses the time evolution of fish abundance throughout the year (from March to February), while a set of difference equations simulates the hatching process which is assumed to occur at the beginning of March. The model takes into account changes in fish abundance due to dispersal (anadromous movements are not considered), reproduction and mortality. Individuals move from node *i* to node *j* according to their age-class and the presence of longitudinal barriers in the network. At the beginning of each year, individuals pass from one age-class to the next (except the adults) and the 0+ class is supplied by newborn individuals generated by adults. Reproduction takes place once a year (see Figure 5.2).

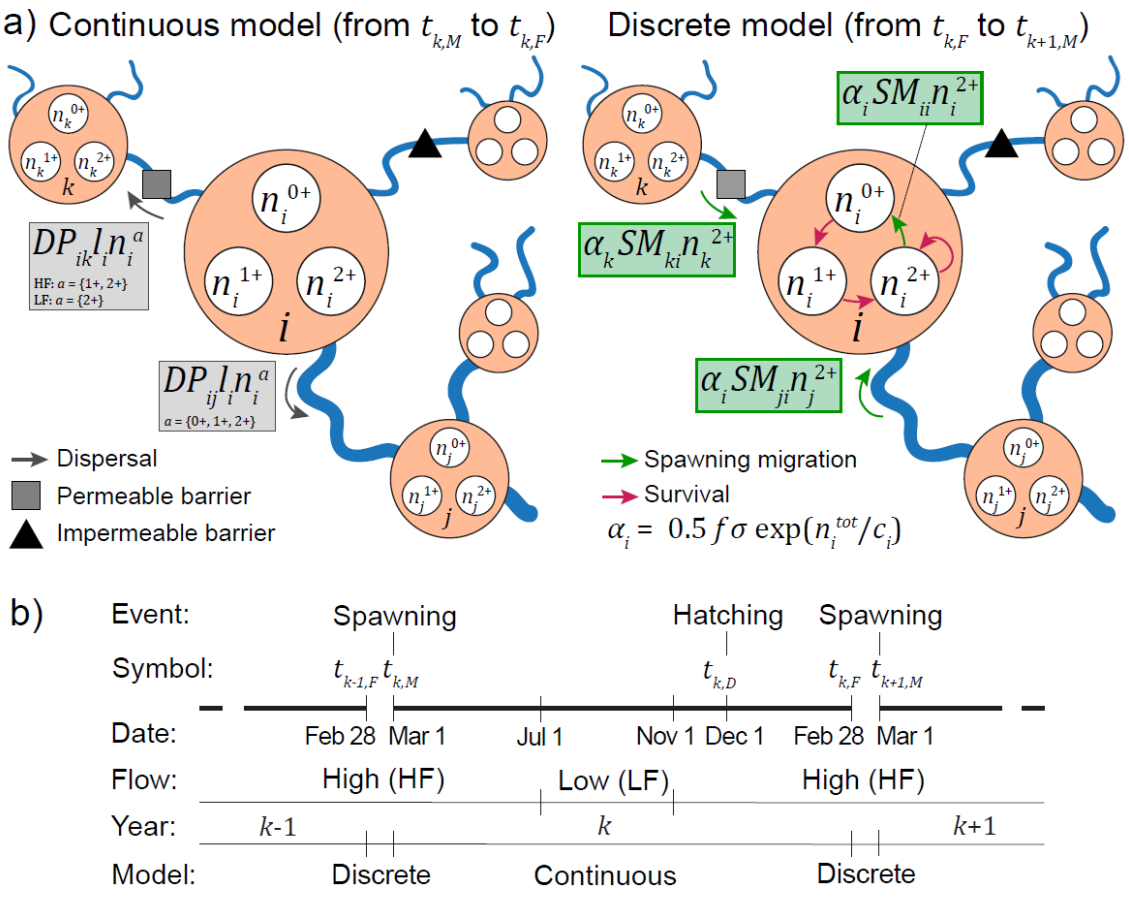

Figure 5.2. a) Schematic representation of the metapopulation model. Natural mortality is not displayed. b) Timeline for the metapopulation model. See section 5.2.3 "Metapopulation model" in the main text for parameters definition and model description.

Continuous model

The continuous model spans from time $t_{k,M}$ (year k, beginning of March M) to $t_{k,F}$ (year k, end of February F) and is constituted by the following set of differential equations:

$$\frac{dn_i^{0+}}{dt} = -\mu^{0+}n_i^{0+} - l^{0+}n_i^{0+} + \sum_{j=1}^{N} DP_{ji}\, l^{0+}n_j^{0+};$$

$$\frac{dn_i^{1+}}{dt} = -\mu^{1+}n_i^{1+} - l^{1+}n_i^{1+} + \sum_{j=1}^{N} DP_{ji}\, l^{1+}n_j^{1+};$$

$$\frac{dn_i^{2+}}{dt} = -\mu^{2+}n_i^{2+} - l^{2+}n_i^{2+} + \sum_{j=1}^{N} DP_{ji}\, l^{2+}n_j^{2+}.$$

where n_i^a is the abundance of individuals for each age-class (a: 0+,1+ or 2+) in the i-th node of the river network; μ^a and l^a are the age-class specific mortality rates and dispersal rates, respectively; DP_{ji} is the dispersal probability, namely the probability that a fish moving out of node j chooses node i as destination. In order to specify the dispersal matrix DP, we first introduce the age-class specific probabilities of downstream (P_d^a) or upstream (P_u^a) movement

$$P_d^a = \frac{1 + b^a}{2}; \quad P_u^a = 1 - P_d^a$$

where b^a is the bias in the direction of movement. The generic entry of the dispersal matrix reads then:

$$DP_{ij}^a = \frac{P_{ij}^a}{\sum_{k=1}^{N} P_{ik}^a}$$

where $P_{ij}^a = P_d^a$ if node i drains into j and there is no impermeable barrier between the two nodes; $P_{ij}^a = A_j P_u^a / \left(\sum_{k \in U_i} A_k\right)$ if j drains into i and in the absence of impermeable barriers (A_j is the drainage area at node j and U_i the set of nodes that drain into i); $P_{ij}^a = 0$ if there is an impermeable barrier between i and j. Note that DP is time dependent: in fact, two different DP matrices are computed, one for the low and one for the high flow condition, as defined below.

Regarding the movement bias b^a, we found contrasting evidences in literature. Some studies argue that the predominant movement is upstream (e. g. Fjellheim et al., 1995; Kahler et al., 2001), while others observed a tendency towards downstream movement (e. g. Mortensen, 1977) or reported no preferences (e. g. Knouft & Spotila, 2002). Moreover, the direction of movement could be influenced by several factors as water flow or temperature (N. Jonsson, 1991). Based on literature, our expert knowledge and information provided by the regional fisheries officers we decided to study two different cases in relation to movement bias for the different brown trout age-classes. The first case (movement bias) assumed that adults present an overall tendency to move downstream to search for more suitable habitats (e. g. Sanz et al., 2011) and to satisfy their metabolic requirements (Cucherousset et al., 2005), while juveniles tend to move upstream motivated by several environmental factors such as movement to feeding areas and away from high-density areas, or avoiding predation (W. H. Pearson et al.,

2001). The other case (movement no-bias) contemplates no-bias on brown trout movement (i.e. same preference to move up or downstream). Thus, the movement bias case was set with the following movement direction preferences: $b^{0+} = 0$; $b^{1+} = -0.2$; $b^{2+} = 0.2$, while in the movement no-bias all parameters were set to 0.

Obstacles were considered as permeable or impermeable (in both directions: upstream and downstream) longitudinal barriers depending on their physical characteristics, the fish age-class and the hydrological regime, following a national standard for the evaluation of fish pass permeability (MAGRAMA, 2015). Hydrological conditions were distinguished between low flow (LF) and high flow (HF) depending on the seasonality, with LF occurring between July and October and HF between November and June (see Figure 5.2b). For age class 2+, permeable longitudinal barriers are obstacles with a maximum height of jump not greater than 1 m, minimum depth of the pool exceeding the height of jump at least by 25%, and maximum width crest not greater than 0.5 m during LF. During HF, the previous metrics are increased by 15%. Any other obstacle was considered permeable, unless it had a non-functional fish pass. For class 1+, all barriers are impermeable during LF. During HF, obstacles are treated as in the case of class 2+. For class 0+, all barriers are impermeable regardless of the hydrological regime. Based on these conditions, 79 out of 139 longitudinal barriers in the study area were permeable for adults and temporarily permeable for juveniles. Barriers locations are shown in Figure 5.1.

Discrete model

The discrete model (from $t_{k,F}$ to $t_{k+1,M}$) is constituted by the following set of equations:

$$n_i^{0+}(t_{k+1,M}) = \sum_{j=1}^{N} 0.5\, SM_{ji} n_j^{2+}(t_{k,D}) f\, \sigma \exp\left(-\frac{n_i^{total}(t_{k,F})}{C_i}\right)$$

$$n_i^{1+}(t_{k+1,M}) = n_i^{0+}(t_{k,F})$$

$$n_i^{2+}(t_{k+1,M}) = n_i^{2+}(t_{k,F}) + n_i^{1+}(t_{k,F})$$

The abundance of newborn individuals at the beginning of the year $n_i^{0+}(t_{k+1,M})$ is the outcome of the spawning and hatching processes which take place during winter. Spawning and hatching dates depend on environmental conditions, and primarily on water temperature (Elliott & Hurley, 1998). In the Deva-Cares catchment, spawning and

hatching activities reach a peak in December and March, respectively (Cantabria Regional Government pers. comm.). Similar spawning and hatching periods have also been found in nearby catchments (e. g. Lobón-Cerviá, 2005). The total number of spawned eggs was estimated with a uniform sex ratio (1:1) as the product of adult females in December (half of the adult population $n_j^{2+}(t_{k,D})$) times the number of spawned eggs per individual (f). The number of newborn individuals is the product of the number of spawned eggs and the hatching probability (σ). Hatching success depends on several physicochemical variables (Sternecker et al., 2013) and can vary among sites and years. The value of σ was set equal to 0.1 based on local experience (Cantabria Regional Government pers. comm.). It is well known that inter-annual variation of recruitment is mainly governed by fry survival during hatching and emergence periods, which, in turn, is driven by flow and temperature conditions (Lobón-Cerviá & Rincón, 2004; Bret et al., 2015). However, this parameter is assumed constant in space and time because we want to simulate long-term stability conditions (average situation) of spatial density patterns at a river network level, rather than temporal dynamics of abundance. This argument is also applied for the other parameters (e.g. mortality rates) that are assumed constant in space and time.

Spawning migration patterns were defined according to a gravity model (Erlander & Stewart, 1990). The probability SM_{ij} that adult brown trout move from node *i* to *j* for spawning and then return to node *i* is proportional to a site-specific spawning suitability score SS_j and inversely proportional to the distance between the two nodes d_{ij} (through an exponential kernel with scale factor *D* which indicates a typical deterrence distance for migration):

$$SM_{ij} = \frac{SS_j \ \exp\left(-\frac{d_{ij}}{D}\right)}{\sum_{k=1}^{N} SS_k \exp\left(-\frac{d_{ik}}{D}\right)}$$

where d_{ij} is set to infinity when nodes *i* and *j* are not connected owing to topology or presence of an impermeable barrier. Spawning suitability scores were calculated as $SS_j = \log(A_j)^{-3}$, as tributaries and headwaters are generally more suitable for spawning (e. g. Gosset et al., 2006).

Several authors have found population density in salmonids to be regulated by density-dependent mechanisms (associated with food availability, competition and spatial relationships) in the early life stages (Elliott, 1989a; B. Jonsson & Jonsson, 2011), especially in locations with favorable habitats with high densities. In accordance to Haldane`s hypothesis (Haldane, 1956), such an endogenous regulation mechanism has been rarely reported in peripheral populations in unfavorable habitats with low density, like the Iberian peninsula. In contrast, external factors (especially extreme flow events during the late embryo developmental stages and early emergence) have been found to locally limit brown trout population density. This phenomenon of recruitment limitation is modelled by means of a Ricker recruitment model (Ricker, 1954) with density dependent factor $C_i = cH_i$, which represents a proxy of the carrying capacity of node *i*, where c is the fish density at carrying capacity and H_i is the habitat size, namely the product of mean water width and length of the node. We assumed $c = 0.83$ ind/m^2, equal to the average maximum density obtained in a sample point in data set FD2. In our simulation the Ricker recruitment model mimics the density-dependent effect of suitable habitats potentially limiting the maximum numbers of successful nests in a given stream (Einum, 2005) while accounting for a well-known environmental limitation of recruitment by stream hydrology (Lobón-Cerviá & Rincón, 2004). Lengths were obtained from the subdivision of the river network into nodes, while mean water widths were obtained with Spatial Stream Network models (Ver Hoef et al., 2014) from empirical data with information on section E of River Habitat Survey (Environment Agency, 2003).

The metapopulation model was run for 50 years, with the aim of reaching a steady spatial distribution of fish densities unaffected by the initial conditions. We will hereafter focus on the results obtained for the 50th year of simulation. The initial presence-absence spatial pattern was derived from data set FD1. We assumed that initial fish densities for 0+ and 2+ age-classes were null, while juveniles' initial densities were set to their value at carrying capacity in the nodes where initial presence had been predicted, and null otherwise.

5.2.3.1 Model calibration

Some parameters were not available for our study area due to the lack of observational data and hence they required calibration. These were mortality rates, dispersal rates,

deterrence distance for spawning migration and the number of spawned eggs per individual. Feasible parameter ranges were obtained from literature.

As for the deterrence distance for spawning migration (D), we used a range between 500 and 9500 m, based on previous studies which show a mean upstream distance for reproduction within that range (e. g. Rustadbakken et al., 2004; Gosset et al., 2006). Regarding mortality rates ($\mu^{0+}, \mu^{1+}, \mu^{2+}$), our model assumes a single value for each of these parameters and does not differentiate between natural, fishing and temperature-dependent mortality rates. In this regard, there are studies that show very different values among brown trout cohorts and populations (Lobón-Cerviá et al., 2012). Thus, we chose a range of mortality values (0.001 to 0.005 d^{-1}) close to a population average value of 0.0025 d^{-1} obtained from a nearby northern Spanish catchment (Esva basin; Lobón-Cerviá et al., 2012). Values of μ^{0+} were set slightly higher because early life stages suffer higher mortality rates (Wootton, 2012). In relation to dispersal, salmonid populations are composed of both stationary and mobile individuals (e. g. Bridcut & Giller, 1993), being the mobile fraction less abundant (Young et al., 2010), or even a very reduced fraction (i.e., less than 5%; Rocaspana et al., 2013). Studies of fish movements have yielded different results based on genetic analysis (e. g. Gowan et al., 1994; Vera et al., 2010), dispersal distances (Young et al., 2010) or dispersal rates (Crisp, 1991). Because dispersal of 0+ age-class trout is limited (Vatland & Caudron, 2015), the distances covered are small (Elliott, 1987) and the average node length is 1000 m, we set $l^{0+} = 0$ d^{-1}. We further assumed that adults will have the highest dispersal rate (Olsson & Greenberg, 2004; Vera et al., 2010) with a maximum of 0.12 d^{-1} and half that value as upper boundary for the 1+ age-class. Fecundity highly depends on fish length (Lobon-Cervia et al., 1997; Nicola & Almodóvar, 2002) or body mass (N. Jonsson & Jonsson, 1999), with the number of eggs ranging between 1000 and 2000 per kg (Doadrio, 2002). The average fecundity for resident trout with weights between 100 and 500 g is between 270 and 1100 eggs (Klemetsen et al., 2003; Alonso et al., 2012). Based on our empirical data (mean ± SD adult fork length: 194 ± 49 mm; mean ± SD adult weight: 97 ± 40 g), we choose values of f from 200 to 400. Parameter ranges are reported in Table 5.1.

The model was calibrated against mean observed fish density data from data set FD2. In particular, calibration was performed on a subset of 13 nodes of the fluvial network where more than one measurement was available. Modelled fish densities (average of

August, September and October) were deemed as appropriate if they were within the 95% confidence interval (CI) of a *t* distribution based on the observed population density for each sample point. We assumed that fish density followed a *t*-distribution because of the low number of replicas per node (between 3 and 6 years). The maximum number of correct results would be 39 (13 nodes times 3 age-classes within the 95% CI), thus, we selected the best-fit parameter set as that that achieved the greatest number of correct predictions for each node and age-class.

Table 5.1. Parameters that require calibration and corresponding ranges. Note that the dispersal rate for young-of-the-year fish $\boldsymbol{l^{0+}}$ was set to 0. (*) Explanation to the value references are given in the text.

Variable	Units	Min. value	Max. value	References(*)	Calibration values
D	m	500	9500	(Rustadbakken et al., 2004; Gosset et al., 2006)	2000
μ^{0+}	d^{-1}	0.002	0.006		0.003
μ^{1+}	d^{-1}	0.001	0.005	(Lobón-Cerviá et al., 2012)	0.002
μ^{2+}	d^{-1}	0.001	0.005		0.003
l^{0+}	d^{-1}	0	0		0
l^{1+}	d^{-1}	0	0.06	(Rocaspana et al., 2013)	0.015
l^{2+}	d^{-1}	0	0.12		0.06
f	-	200	400	(Doadrio, 2002)	340

5.2.4 Simulations of scenarios

The metapopulation model was used to detect how modifications to river network connectivity due to removal of longitudinal barriers influenced patterns of brown trout population density. Three different scenarios generated by changes in network connectivity were explored. Scenario 1 refers to the current situation of the river network where all longitudinal barriers are present and connectivity is restricted. This scenario mainly serves as a baseline for comparison with the other scenarios. Scenario 2 explores the condition of complete connectivity, were all longitudinal barriers are removed. Finally, scenario 3 represents a situation with improved local connectivity, where a single impermeable barrier is removed. We selected one of the highest barrier (dam height = 15 m) located in the Picos de Europa Natural Park which is important because it limits the distribution of *Salmo salar* (Linnaeus, 1758), and therefore of

brown trout population. We consider that this barrier limits both species since swimming performance curves for salmonids are given as a function of their length, not of the species (Larinier (2002) based on Beach (1984)). This barrier isolates a catchment area of 266 km^2 and affects a total upstream river network length of around 100 km. Due to the lack of clear management scenarios in the Deva-Cares catchment, we consider that this selection could illustrate the effects of restoring longitudinal connectivity on a single river network section.

5.2.5 Sensitivity analysis

To understand how model parameters affect density results, we performed a one factor at-a-time sensitivity analysis of the model outcomes with respect to dispersal parameters (l^{2+}, l^{1+} and D) focusing on scenario 1 and 2 and the assumption of bias/no-bias. Although the rest of parameters (e.g. mortality rates) would have a direct effect on the overall density, we only performed the sensitivity analysis on parameters related to movement due to our interest in the effects of movement bias in relation to river network connectivity.

Maps showing the obtained results were developed for the different scenarios using ArcGIS 10.2 (ESRI, 2014). All models were run using MATLAB 8.5.0.197613 (R2015a) (MATLAB, 2015). Statistical analysis were performed in the R 3.1.3 software (R Core Team, 2015) and the editor RStudio (RStudio, 2015).

5.3 Results

5.3.1 Metapopulation model

The model parameters that obtained the best fit with empirical data (see Table 5.1) yielded 29 model results (11 for 0+, 9 for 1+ and 9 for 2+) within the 95% CI of the empirical data (see Figure 5.3).

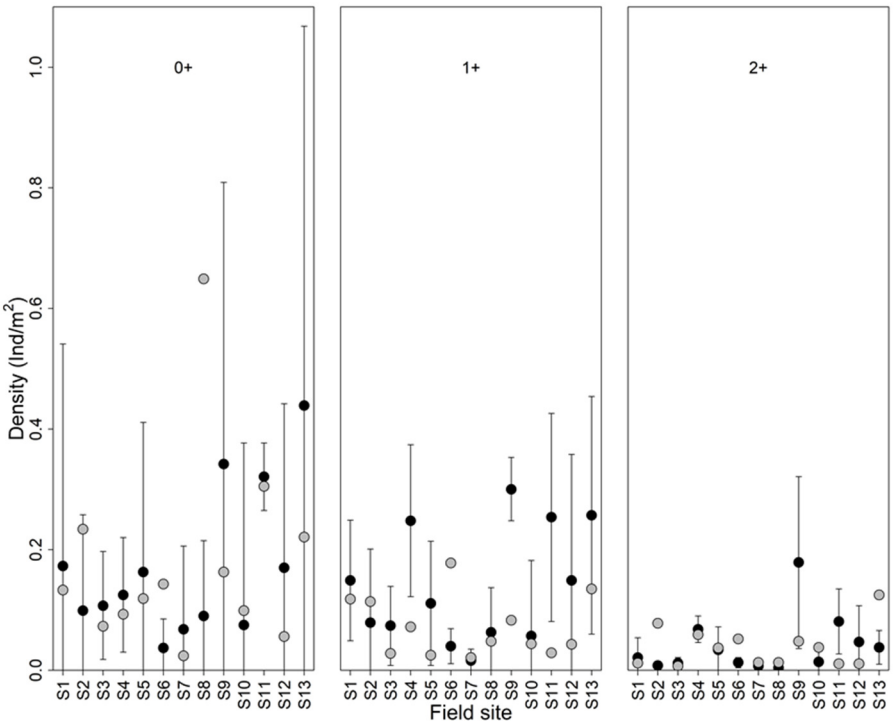

Figure 5.3. Modelled densities (grey circles) and average densities observed in the field across years (black circles; see section 5.2.2: FD2) for three different brown trout age-classes in 13 river reaches of the Deva-Cares catchment. Bars show 95 % confidence intervals of average observed densities based on a *t* distribution.

5.3.2 Simulation of scenarios

Within scenario 1, density decreased with increasing age-class (Figure 5.4). Generally, modelled density of 0+ was higher in the headwater reaches and density of 1+ and 2+ was higher in the middle river reaches (see also Figure 5.5; scenario 1). The network-averaged fish densities (total number of fish divided by the habitat size) were 0.171 ind/m^2 (0+), 0.064 ind/m^2 (1+) and 0.041 ind/m^2 (2+) considering bias movement (see Table 5.2). Bias movement had a moderate effect on fish density for each age class, observing a decrease of less than 10% in relation to the no-bias assumption.

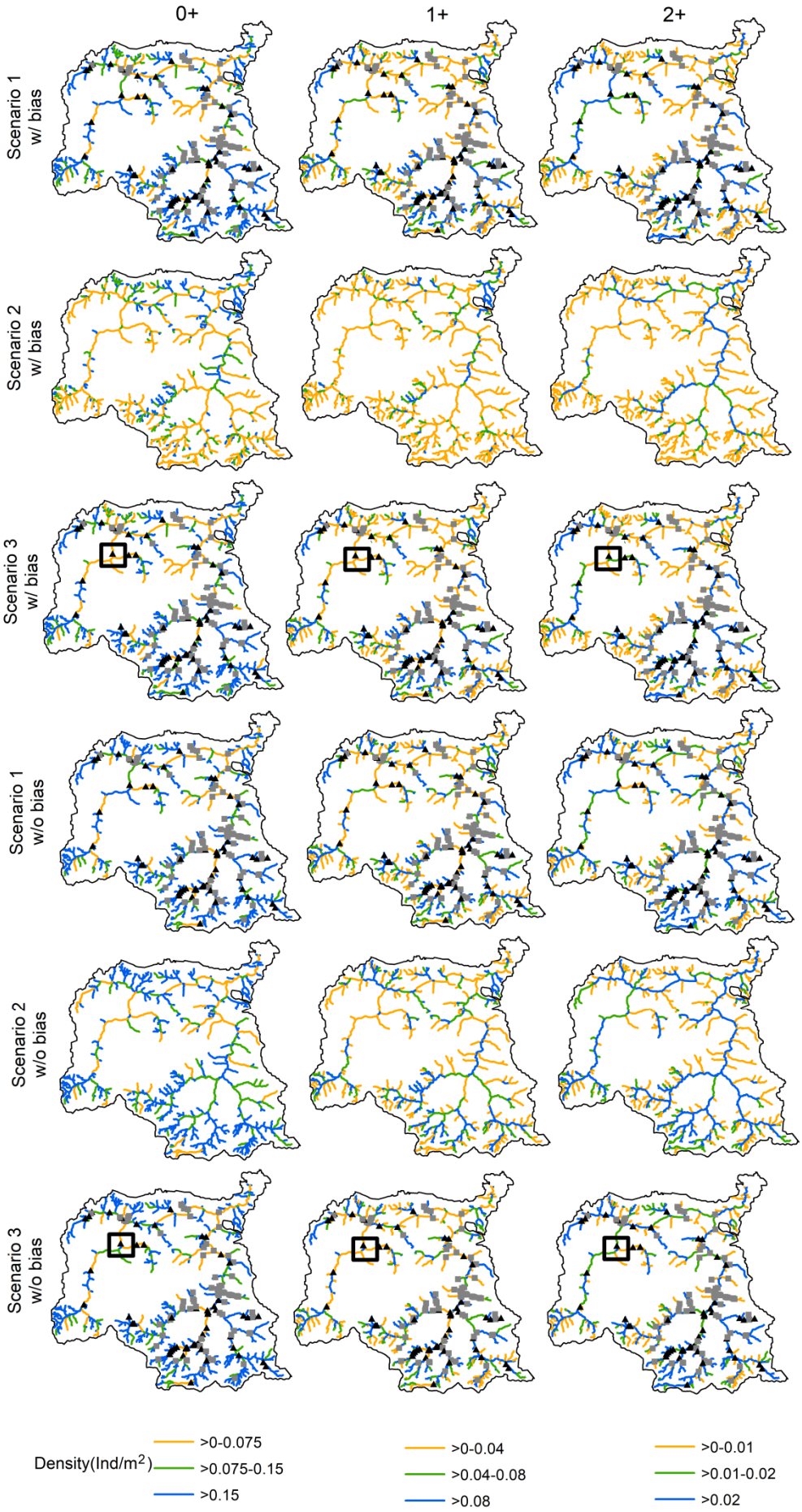
0+
1+
2+
Scenario 1 w/ bias
Scenario 2 w/ bias
Scenario 3 w/ bias
Scenario 1 w/o bias
Scenario 2 w/o bias
Scenario 3 w/o bias
Density(Ind/m2)
>0-0.075
>0.075-0.15
>0.15
>0-0.04
>0.04-0.08
>0.08
>0-0.01
>0.01-0.02
>0.02

Figure 5.4. Modelled fish density (individuals/m^2) for different age-classes, scenarios and inclusion or neglect of movement bias. In scenario 3, the removed longitudinal impermeable barrier is shown within a black rectangle.

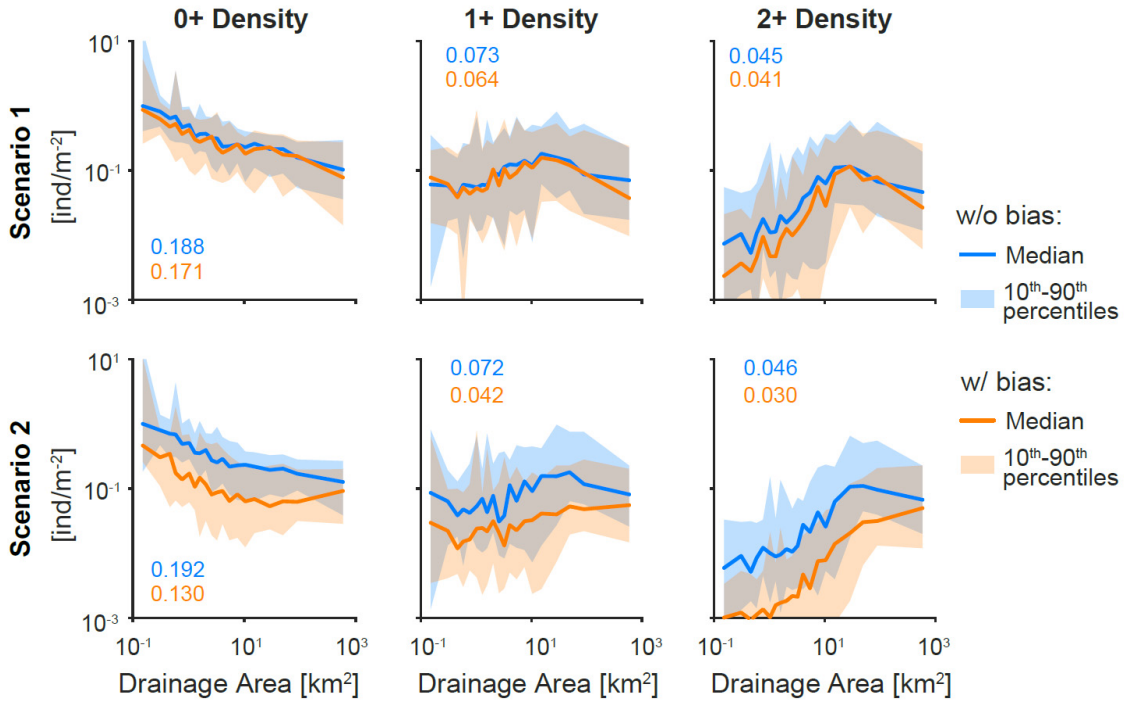

Figure 5.5. Distribution of fish density across all nodes as a function of drainage area. Rows identify scenarios, columns refer to age classes and colors represent inclusion or neglect of movement bias. Displayed values of fish density are the averages during August, September and October of the last year of simulation (steady state) obtained with the calibrated set of parameters of Table 5.1. The displayed percentiles are obtained through a binning procedure: each data series is sorted in ascending order in terms of drainage area and then divided in 20 equal parts; percentiles are then plotted at the midpoint of each bin. Colored numbers within each subplot refer to the overall fish density (in ind/m^2) for the respective combination of scenario, age class and assumption on bias.

For scenario 2 and no-bias, the patterns of fish density were similar to the no-bias case of scenario 1 (see Figure 5.4 and Figure 5.5; network-averaged fish densities were 0.192 ind/m^2 (0+), 0.072 ind/m^2 (1+) and 0.046 ind/m^2 (2+)). However, when bias movement is included, the decay in fish density is remarkable compared to the no-bias case in scenario 1 (see Table 5.2). In the absence of longitudinal barriers, modelled density of 0+ class is higher in river reaches with small catchment areas, while 1+ and 2+ age-class show higher densities in nodes with larger catchment area (Figure 5.5). Although this is also the case in scenario 1, the pattern is more pronounced in the complete

connectivity scenario (Figure 5.5). Overall, brown trout population density under scenario 2 tends to decrease in the smaller tributaries, while it increases in the lower part of the catchment (Figure 5.5 and Figure 5.6). In this scenario, the network-averaged mean densities are 0.130 ind/m^2 (0+), 0.042 ind/m^2 (1+) and 0.030 ind/m^2 (2+), namely 24%, 35% and 25% less compared to scenario 1, respectively. It is remarkable that under scenario 2 and bias movement most river network nodes showed important changes (more than 50% increases or decreases) on fish population densities in comparison to scenario 1 (Figure 5.6). When no-bias movement is considered, most river network nodes also showed changes in comparison to the analogous case scenario 1, tending to increase the population density in the main channels (Figure 5.6), however, the network-averaged mean densities are only 1% (1+) and 2% (0+ and 2+) lower compared to scenario 1.

Table 5.2. Network-averaged mean densities (ind/m^2) for the respective combination of scenarios, age class and inclusion or neglect of movement bias (w/bias and w/o bias). Values of scenario 1 for the same nodes (upstream and downstream) were the changes are produced in scenario 3 are also represented.

	0+		1+		2+	
	w/ bias	w/o bias	w/ bias	w/o bias	w/ bias	w/o bias
Scenario 1	0.171	0.188	0.064	0.073	0.041	0.045
Scenario 2	0.130	0.192	0.042	0.072	0.030	0.046
Scenario 3 (upstream)	0.010	0.084	0.006	0.034	0.002	0.022
Scenario 1 (upstream)	0.180	0.153	0.075	0.063	0.044	0.037
Scenario 3 (downstream)	0.107	0.158	0.032	0.056	0.025	0.037
Scenario 1 (downstream)	0.102	0.143	0.032	0.052	0.024	0.034

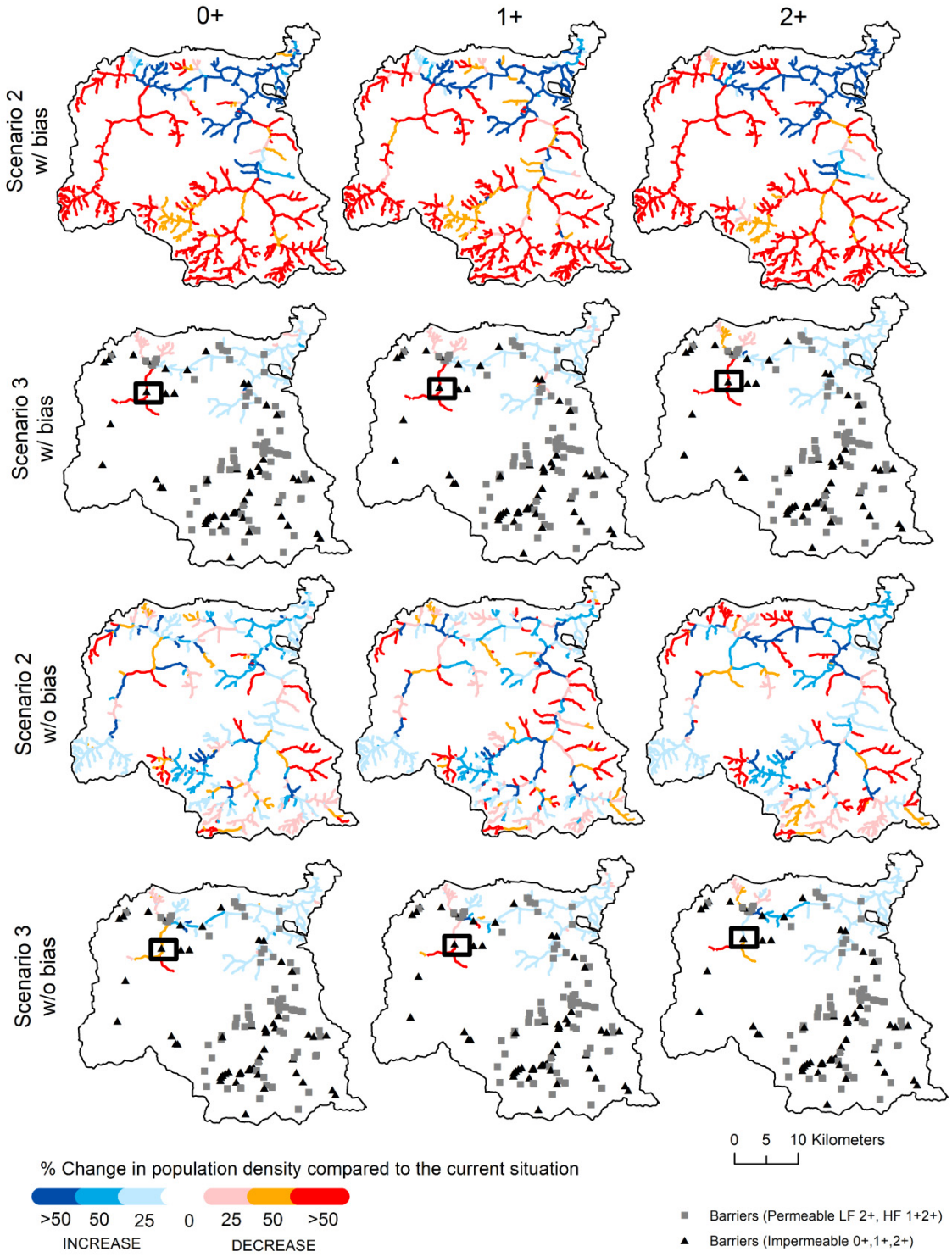

Figure 5.6. Percentage of change in the modelled population density for scenario 2 and scenario 3 compared to scenario 1 with inclusion and neglect of movement bias. In scenario 3, the removed longitudinal impermeable barrier is shown within a black rectangle.

Scenario 3 with movement bias (Figure 5.4 and Figure 5.6) featured a decline in population density (mean densities: 0.010 ind/m^2 for 0+, 0.006 ind/m^2 for 1+ and 0.002

ind/m^2 for 2+ age-classes) upstream of the removed barrier, while an opposite trend was observed downstream (mean densities of 0.107 ind/m^2 for 0+, 0.032 ind/m^2 for 1+ and 0.025 ind/m^2 for 2+ age-classes). These changes represent an average percentage decrease of 94% upstream and of 4% increase downstream (Figure 5.5). Overall, scenario 3 produced a decrease of less than 5% in the network-averaged density. However, it is remarkable that the effect of the local dam removal extends till the next longitudinal impermeable barrier in either both or downstream directions. When no-bias movement is considered the mean densities follow a similar pattern in regard to scenario 1 for the same nodes decreasing upstream of the barrier but increasing downstream (see Table 5.2). In this case, upstream decrease is lower (44%) and downstream increase is greater (10%) than when assuming movement bias.

5.3.3 Sensitivity analysis

As l^{2+} increased (central column; Figure 5.7), the differences between the overall fish densities (for all age classes) calculated with and without bias increased. When considering no-bias movement, overall fish densities were not much sensitive to changes in this parameter, as high l^{2+} in this case only increased the speed at which adults move, but the spatial distribution of density was not affected. Instead, when bias was included, high values of l^{2+} enhance the downstream tendency of adult fish, making them less likely to spawn in the upstream reaches (especially in scenario 2, where there is no obstacles to downstream movement).

Increases in l^{1+} (left column; Figure 5.7) caused an increase in the overall fish density (for all age classes), in particular when movement bias was included. In this case, the tendency of juvenile fish to move upstream is enhanced, and this contributed to push the adult fish towards more upstream reaches. On the other hand, the effects of high l^{1+} when no-bias movement was considered were minor. The very high overall fish density for class 0+ when l^{1+} approaches 0 (top left panel; Figure 5.7) is explained in the Supplementary material 5.

When D is set to high values (right column; Figure 5.7), the effect of movement bias tends to disappear. It is remarkable that, in this case, the overall fish density was a bit higher for scenario 1 than for scenario 2, regardless of the assumption on movement

bias. This implies that there is an effect of reduced connectivity in increasing the overall fish density beyond the bias/no bias movement issue.

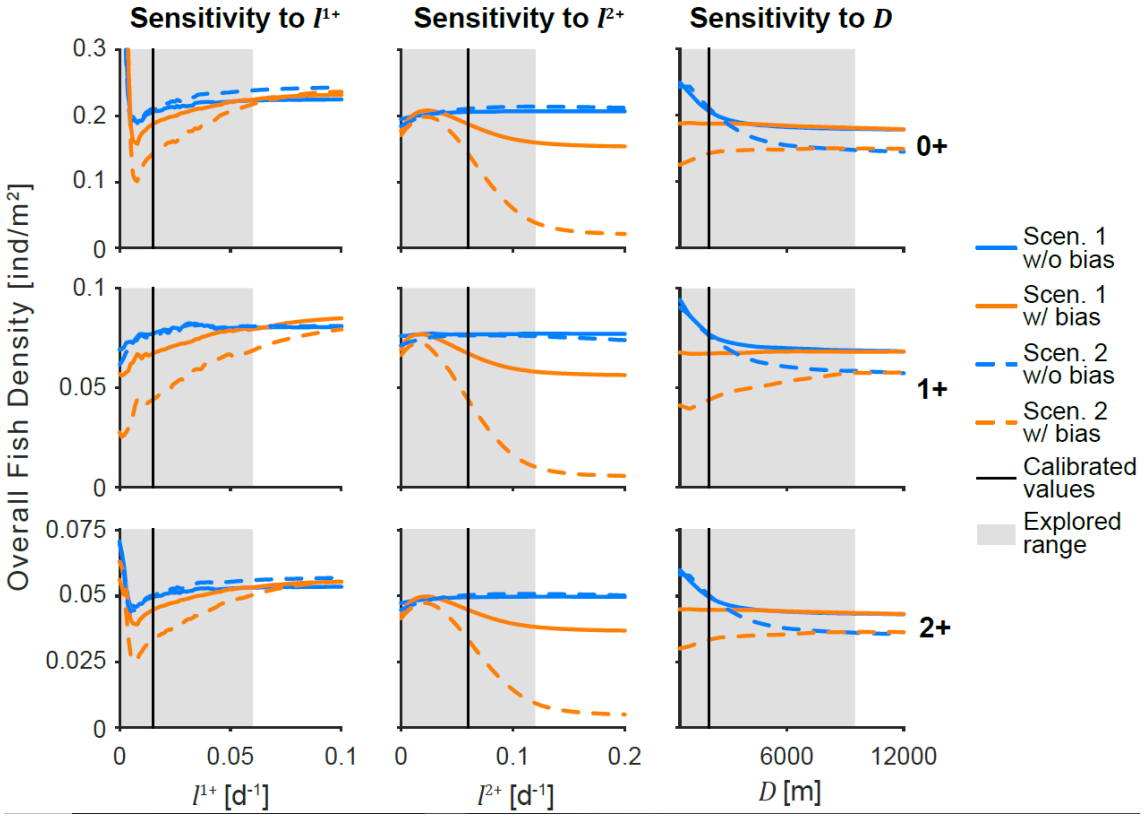

Figure 5.7. Results from sensitivity analysis. Effect of variations of some meaningful parameters (columns) on overall fish density for the three age classes (rows) for different scenarios (line type) and inclusion/neglect of movement bias (line color) Black solid lines identify calibrated parameter values from Table 5.1. Gray shaded areas highlight explored parameter ranges in calibration (see Table 5.1). Displayed values of fish density are the averages during August, September and October of the last year of simulation (steady state), where all parameters not tested for sensitivity were kept to their calibrated value (Table 5.1).

5.4 Discussion

5.4.1 Metapopulation model

Obviously, there exists a vast literature on successful models of fish (especially salmonids) metapopulations that describes the spatial effects that river network connectivity exerts on demography and genetics of populations (see e.g. Labonne et al. (2008) and Muneepeerakul et al. (2007) for abstract networks, Fagan (2002) and

Muneepeerakul et al. (2008) for real-life settings and Fullerton et al. (2016) for applied/management purposes). As stream ecosystems prove sensitive to connectivity and segregation regardless of many details of the relevant ecology (Muneepeerakul et al., 2008), and given the number of specific studies on brown trout's ecology, the present work builds on related studies, including a realm of specific field data, to support its general findings, using the actual connectivity of the test catchment and field evidence to calibrate the model parameters that affect local extinctions and/or the size of the metapopulation. Thus, the metapopulation model proposed in this study is one of the first models that estimates the spatial patterns of density of native brown trout at a river network scale considering the real topology and using empirical data for the calibration. Our results produce an appropriate representation of the actual spatial variability of brown trout densities for the three age-classes, as reflected by the adjustment of the modelled results to the empirical data (i.e. 75% overall agreement). Moreover, when removing all longitudinal barriers the spatial pattern of fish densities tends towards the expected spatial pattern for a natural river network, with higher densities of adult trout on the downstream portion of the network and higher densities of younger individuals upstream (Baglinière & Maisse, 1999). However, we need to acknowledge that these modelled results are a direct consequence of a number of different assumptions and issues.

First, the absence of other important environmental or anthropogenic drivers of fish densities in the case study catchment besides connectivity provides a suitable scenario to apply the proposed model. Indeed, our model was developed in a catchment with a river network composed by perennial reaches (González-Ferreras & Barquín, 2017), but this situation is not characteristic of other catchments where the presence of dry reaches or disconnected pools alters longitudinal connectivity (e.g. Larned et al., 2011) and population dynamics (Perry & Bond, 2009). Moreover, we did not consider anadromous movements because the size of the anadromous population in our study area is small. Thus, the results might not hold in other catchments where other environmental or anthropogenic drivers (e.g. exotic species, sewage discharges, etc.) control the spatial patterns of brown trout densities (e.g. Elvira & Almodóvar, 2001; Leunda, 2010).

Second, we made some assumptions related to key population characteristics of the species (e.g. movement preference downstream or upstream, sex ratio, etc.) based on previous knowledge and literature review. However, these characteristics were not

specifically assessed in the local population. Thus, the relevance of these assumptions cannot be evaluated and the applicability of the current model calibration might not be appropriate for other studies in different catchments. This is especially relevant in the case of brown trout as this species shows a high phenotypic plasticity (B. Jonsson & Jonsson, 2011), with remarkable differences among different populations. In this regard, we acknowledge that the chosen values of the population dynamic variables (mortality rates, dispersal rates, deterrence distance for spawning migration, etc.) are constant in space, although several studies showed that these variables could change within and among populations. For instance, mortality rates are affected by many factors (age, size, sex, environmental factors, fishing, etc.; (Elliott, 1993)) and they could be different among river reaches of the same catchment (Lobón-Cerviá, 2012). In particular, we believe that fishing might contribute to the high mortality rate of adults relative to young-of-the-year individuals, although further investigation on this aspect would be required.

Improvements to the current modelling approach should take into account the spatio-temporal heterogeneity of the population dynamic variables, but this also implies that more data on local populations would be necessary. Our study is focused on general spatial trends, but the inclusion of this information could yield further insights on spatio-temporal population dynamics. For example, dispersal rates can vary between water velocities (Crisp, 1991) or seasons (Ovidio et al., 2002). Dispersal rates also depend on carrying capacity, although density-dependent dispersal rate was not included in the model because densities are generally low and carrying capacity is seldom reached. Still, the inclusion of density-dependent dispersal will be an enhancement of the model. Moreover, temporal fluctuations of environmental variables are also important in determining changes in fish densities; thus, our model could be enhanced by including seasonality and, possibly, stochasticity (Borsuk & Lee, 2009) to assess the effects of spatio-temporal heterogeneity in environmental conditions. Thus, stochasticity could be incorporated by relating flow regimes to mortalities through the existence or not of refuges (hydraulics and suitability changes to flood or droughts) in each node. This approach would allow the user to analyze how climate change (e. g. Almodovar et al., 2012) or hydrological alterations (e. g. Sabater & Tockner, 2010) might affect spatiotemporal patterns of population dynamics, and it should constitute an improvement of the actual modelling framework with results in the near future.

5.4.2 Simulation of scenarios

River networks can be affected by local or macroscale phenomena (McCluney et al., 2014). However, because of the longitudinal connectivity of river ecosystems, the propagation of changes in fish density is particularly sensitive to the location where an alteration occurs in the network (Charles et al., 2000; Thorp et al., 2013; Samia et al., 2015). In the present study, the removal of all longitudinal barriers (scenario 2) produced a decline of more than 25% in the network-average brown trout density considering the inclusion of movement bias. However, this result should be interpreted with caution; when no movement bias is considered the network-average densities are similar for both scenarios. However, sensitivity analysis on *D* parameter showed an effect of reduced connectivity in increasing the overall fish density beyond bias/no-bias movement (i.e. increasing *D* values makes the bias-no bias movement cases converge, but scenarios 1 and 2 do not converge for all age-classes, showing higher values of density in the scenario 1). This revealed the effect of connectivity on spatial patterns of density and showed that longitudinal barriers could be functioning as possible biomass "traps", as we explain below. Moreover, scenario 2 produced a general increase in brown trout densities downstream and a decrease upstream in relation to the initial situation of the Deva-Cares catchment (scenario 1). In addition to the effect caused by movement bias (see sensitivity analysis), it is possible to explain this pattern as larger brown trout do not found suitable habitat in smaller headwaters as they grow and become larger, which indices them to migrate downstream. Larger brown trout produce and lay larger quantities of eggs (Doadrio, 2002) and in absence of barriers, they will move downstream increasing not only adult densities but also the densities of other life cycle stages, since they spawn on many low order streams directly draining into high order streams. On the contrary, barriers can act as "traps" not allowing the movement of larger brown trout to move to more suitable habitats dowsntream (e.g. deeper main river; Sanz et al., 2011) increasing the densities of all life cycle stages in headwaters or middle reaches "locked" by longitudinal barriers. Therefore, the current lack of longitudinal connectivity might not be sustaining an artificially higher density of brown trout, but rather locally altering the spatial distribution of age class densities. The removal of longitudinal barriers restored brown trout densities to the expected natural pattern, with increasing densities of adults in the main river course and higher young-of-the-year densities in the smaller tributaries. Such a trend has been shown in other

studies (e.g. the Scorff basin in France; Maisse & Bagliniere, 1990; Baglinière & Maisse, 2002). This natural spatial variability pattern has been traditionally attributed to changes in physical habitat characteristics, such as water depth, water velocity, substrate particle size or cover, that produce a spatial zonation of the age-classes (Heggenes et al., 1999). In this regard, studies on ecohydraulics have shown that larger trout select deeper stream areas while younger trout inhabit shallower areas (Heggenes et al., 1999; Ayllón et al., 2009). Most of the relevant physical habitat attributes for the brown trout change longitudinally in a river network, usually scaled via power law relationships with discharge (or a proxy like drainage area; Leopold & Maddock Jr, 1953; downstream hydraulic geometry framework). Although these habitat attributes were not explicitly taken into account in the present study, they were implicitly considered when movement preferences of each age class were parameterized. Moreover, in the case of a single barrier elimination (scenario 3) patterns obtained with bias/no-bias movement are similar (i.e. decrease upstream and increase downstream; although with different densities). It could be necessary to deeply analyze this behavior in future studies by removing other barriers in the network in order to assess which is the effect of the river network position. In this study, we decided to simulate the elimination of all barriers as an extreme scenario (although currently not a feasible option) and the elimination of one single barrier (see section 5.2.4 Simulation of scenarios in the text for explanation) as a more feasible management option, but other barriers could potentially have a different effect.

In the three simulated scenarios (even with and without bias movement), the spatial distribution and densities of the three age-classes changed substantially in many river reaches (more than 50% increases or losses). Loss of connectivity tends to increase population density upstream, while movement bias locally affected or increased population density downstream. However, the construction of realistic scenarios requires a better knowledge on the overall movements of the different age classes through the river network (e.g. telemetry; Höjesjö et al., 2007). This is very important in the case of a keystone species or top-predator such as the brown trout, as such changes on its densities might generate considerable top-down effects on biological communities and ecosystem processes. For example, the decline in Yellowstone cutthroat trout abundance (from 3.5 million in 1979 to 1.7 million in 1997) engendered by the introduction of lake trout altered the lower trophic levels via a trophic cascade

(Koel et al., 2007). Zooplankton biomass was doubled, while phytoplankton biovolume was three times lower in 2004 compared to the period before the introduction of the lake trout (Tronstad et al., 2010). Moreover, field experiments performed in Japan by Nakano et al. (1999) showed that increasing the intensity of Dolly Varden trout predation on benthic aquatic invertebrates resulted on periphyton biomass increases of about one-third. Other examples of top-down effects are the introduction of certain trout species around the world replacing native fishes as the main predators. In this regard, introduced rainbow trout in high-Andean tropical streams reduced by half the densities of certain invertebrate taxa in the benthos (*Andesiops*, Orthocladiinae and Simuliidae sp.) (Vimos et al., 2015). Introduced brown trout in New Zealand streams also generated top-down cascading effects doubling benthic chlorophyll *a* (McIntosh & Townsend, 1996). Thus, the current situation of river connectivity alteration in the Deva-Cares catchment is generating important changes on the spatial patterns of a key species density that, in turn, might disrupt natural river biodiversity patterns and ecosystem functioning. We believe that this might be also the case in many other river networks all over the world in which natural longitudinal connectivity has also been altered (see Nilsson et al., 2005). However, the expected effects of dams might not be the same in all systems, but depend on the local conditions and the purpose of the dams. For instance, an increase in brown trout density has been detected in the originally barbell dominated fish community downstream Itoiz dam in a nearby catchment (Irati river, Navarra, Spain) [unpublished data], where the irrigation scheme is currently preventing severe spates and droughts that characterize the natural regime of this Mediterranean river. Improving our understanding of how changes in key species densities affect river biodiversity and functioning patterns is paramount to improve river management and conservation strategies.

5.5 Conclusions

In conclusion, this study suggests that the proposed empirical data-driven metapopulation model is an appropriate tool to estimate the spatial patterns of age-specific brown trout density in a whole river network and to assess the effect of changes in connectivity. Spatial heterogeneity in fish density is highly dependent on connectivity and population dynamics, although future studies are needed to learn more about parameters related to brown trout movement. Altering the natural river connectivity

results in major changes in the spatial distribution of fish. Removing a single obstacle might have consequences on very distant tributaries, while removing all obstacles will restore brown trout density levels to a more natural pattern. Such results help us understand how variations in key species distribution affect biodiversity in lotic environments, in the perspective of implementing conservation strategies.

5.6 Supplementary material 5

This Supplementary material contains the Figure S5.1 referred to in the main text of the Chapter V.

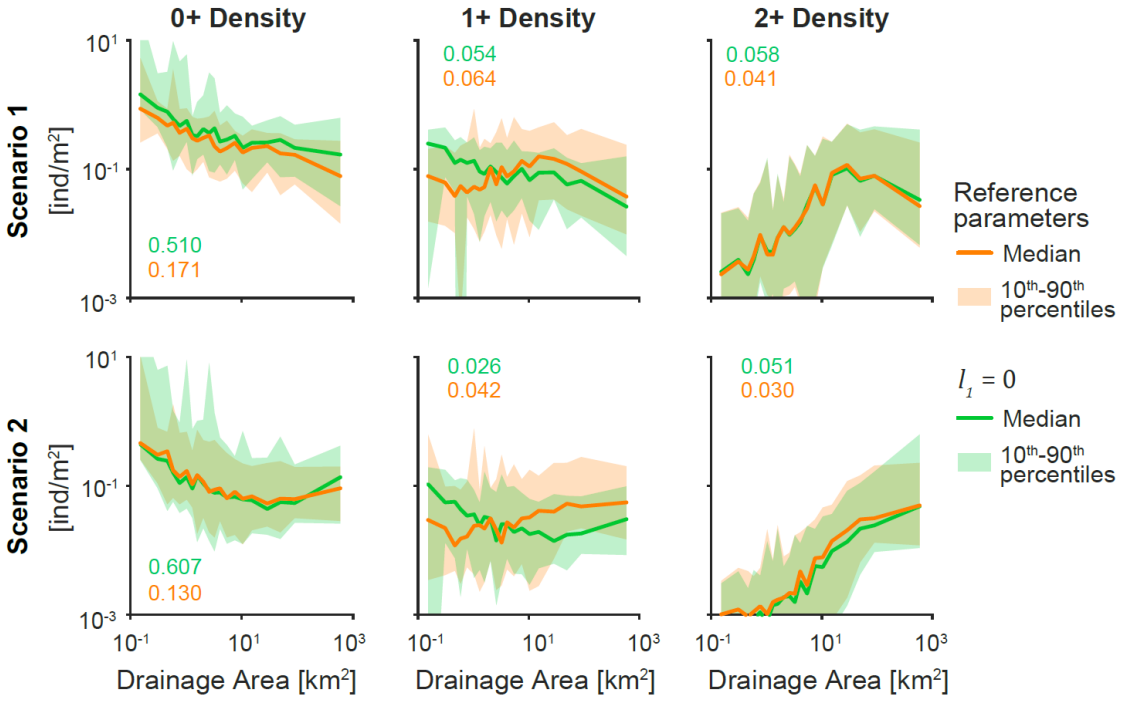

Figure S5.1. Effect of assumption of no fish mobility for juvenile fish in the distribution of fish density as a function of drainage area. Displayed percentiles are obtained as described in Figure 5.7 of the main text. Orange refers to the calibrated set of parameters and the inclusion of movement bias (that is, the same patterns shown in Figure 5.7 of the main text); green refers to the case when bias is included and all parameters are set to their calibrated values, except l^{1+} which is set to zero.

When $l^{1+} = 0$, the distribution of fish density for the juveniles resembles that of the young-of-the-year fish. Therefore, adults are, at the start of the season, close to nodes with high spawning scores. During the season, they tend to move due to $l^{2+}>0$, but they are still closer to headwaters than in the case where they were moving around as juveniles. Hence, adults fish are more likely to spawn again in the upstream sites, which appear to have very high densities of 0+ fish.

Chapter VI

Genetic evidences and consequences of river network connectivity on a native *Salmo trutta* population

Chapter VI: Genetic evidences and consequences of river network connectivity on a native *Salmo trutta* population

This study, performed by González-Ferreras, A.M, Leal, S., Barquín, J. and Almodóvar, A. is under preparation to be submitted for publication in a SCI journal.

Abstract

Network connectivity is a key feature of rivers that affects patterns and processes in these ecosystems. The alteration of connectivity in fluvial networks is important for population and genetic dynamics of aquatic species. Exploring the effects of network fragmentation through genetic analysis is crucial to assess the conservation status of key riverine species. In this study, we investigated the genetic consequences of altered connectivity on a native brown trout population in the Deva-Cares catchment (Northern Spain; 1200 km^2). We investigated i) the genetic variability of brown trout in the catchment, (ii) the genetic differentiation and population structure, (iii) the historical migration rates and the effective population size and (iv) the causes of genetic differentiation and riverscape characteristics. Analysis of genetic variation among 197 individuals from 13 locations covering the whole river network revelated a great level of population differentiation (F_{ST} = 0.181). Below-barrier populations showed high levels of genetic diversity and lower F_{ST} values, while headwater and above-barrier populations showed low levels of genetic diversity and high F_{ST} values. The genetic groups identified were separated by one or more impermeable barriers. We reported a decrease in genetic variability in upstream sites and a downstream-biased gene flow possibly caused by fragmentation, since the results from historical migration indicate that gene flow between all pairwise comparisons was symmetric. Isolation by impermeable barriers played a more important role than hydrological distance on determining the genetic structure of the populations in the Deva-Cares catchment. Most of the populations showed small effective population size, which could lead to an intense genetic drift and higher probability of population extinction. The genetic analysis at the river network scale provide evidence for the role of barriers on determining genetic diversity patterns, highlighting the importance of maintaining and

restoring the river longitudinal connectivity for conserving healthy and diverse brown trout populations.

6.1 Introduction

Rivers are dynamic and hierarchical ecosystems interacting along four dimensions (longitudinal, vertical, lateral and time; Ward, 1989), in which connectivity is considered one of the fundamental properties determining biodiversity and ecosystem functioning patterns (Moore, 2015; Tonkin et al., 2018). From a hydrological perspective, connectivity is defined as "water-mediated transfer of matter, energy, or organisms within or between elements of the hydrological cycle" (sensu Pringle, 2001). The alteration of connectivity in dendritic riverscapes is of great importance for population and genetic dynamics of riverine species (Fagan, 2002; Campbell Grant et al., 2007) due to the branching structure, nested watersheds and stream segments connected by flow in river systems (Fagan, 2002).

Although river networks are naturally fragmented longitudinally (e.g. waterfalls) and laterally (e.g. gorges; Tonkin et al., 2018), human actions have further divided these ecosystems (Fuller et al., 2015) and fragmentation of rivers is growing (Nilsson et al., 2005). One of the most important human impacts in this regard is the presence of longitudinal barriers affecting longitudinal connectivity, which may be physical (e.g. dams), chemical (e.g. pollution plumes), biological (e.g. diseases) or thermal (e.g. stream temperature) and which may change in permeability, location and abundance (Fuller et al., 2015). Fragmentation of river networks by longitudinal barriers can potentially alter population dynamics of aquatic organisms by reducing upstream and downstream migration, changing water quality or habitat conditions (Poff & Hart, 2002). Moreover, fragmented ecosystems might alter evolutionary processes and genetic variation in connected populations by directly influencing gene flow and genetic drift (Dixo et al., 2009). These genetic consequences can make populations more vulnerable to stochastic events because long-term persistence of populations depends on sufficient genetic diversity to adapt and survive in changing environments (A. R. Hughes et al., 2008)

A landscape genetic approach employing geographical landscape and population genetics has become an efficient tool to investigate the effects of landscape

characteristics on population connectivity (Stéphanie Manel et al., 2003). In river networks freshwater species gene flow is constrained by a number of factors, such as catchment geomorphology, the dendritic structure of the river network (Chaput-Bardy et al., 2008) and by species dispersal traits (Tonkin et al., 2018). Fragmentation leads to a decline in gene flow, causing a loss of genetic diversity, increasing inbreeding and increasing risk of local population extinctions (Keyghobadi, 2007; Pavlova et al., 2017). The permeability of longitudinal barriers to gene flow (which is often not available or difficult to evaluate) and the degree of connectivity can determine the severity of the effects on the genetic structure of the riverine populations. Even in the absence of longitudinal barriers, distance itself is also likely to have an effect on connectivity and some populations can be isolated by distance (Wright, 1943) and gene flow decreases with distance among locations (e.g. Sotola et al., 2017).

Organisms that disperse through water (e.g. fish) are likely to be more influenced by the structure of the river network than those that disperse overland (e.g. insects; Tonkin et al., 2018). Most fish species need to occupy multiple habitats over their life cycle (spawning, feeding, etc.), and migratory fishes cover long distances. Thus, river network structure and the loss of connectivity might be more important to fish species (Sheer & Steel, 2006) than to other riverine organisms. Salmonid species are often structured into distinct populations and are considered interesting models for investigating the effects of habitat fragmentation on population structure and evaluate the influence of riverscape characteristics on their genetic structure (e.g. Neville et al., 2006). Within-river population structure of salmonids populations have been explained by contrasting evolutionary models: member-vagrant (Primmer et al., 2006), metapopulation (Dunham & Rieman, 1999), mix of both (Garant et al., 2000) or panmixia (Addison & Wilson, 2010). Member-vagrant model proposes that spawning areas are key determining population structure and local adaptation and selection favors individuals that return to their natal spawning areas where individual that complete the process are considered members and those that do not return are considered vagrants. Evolution is indicated by a strong and temporally stable pattern of population genetic structure with a clear isolation by distance (Garant et al., 2000). Metapopulation hypothesis proposes a set of local populations largely independent but interconnected by migration and subjected to extinction-recolonization processes (Hanski, 1998). Evolution is indicated by a weak genetic differentiation, absence of temporal stability

and absence of isolation by distance (Garant et al., 2000). Panmixia is the absence of a genetic structure due to the existence of gene flow and an unrestricted spawning migration (Griffiths et al., 2009). Information about the evolutionary model which explains genetic differentiation in salmonids populations is important to understand how river network fragmentation and connectivity influence population persistence.

One of the most important salmonids due to its high ecological and socio-economic value is the brown trout (*Salmo trutta,* Linnaeus, 1758). The brown trout is one of the most widespread fish species in European inland waters and its natural distribution is fundamentally restricted to this area (Elliott, 1989c), although it has been introduced in numerous countries around the world. Brown trout populations are composed of both stationary and mobile individuals (Aparicio et al., 2018) and exhibit a very plastic behavior, with different population types (lake-dwelling, stream-resident and sea-migrating) coexisting in the same river network (Klemetsen et al., 2003). The ecology and population dynamics of brown trout has been studied for many years (Elliott, 1994; B. Jonsson & Jonsson, 2011) and several studies on population genetics have been conducted in different regions (Massa-Gallucci et al., 2010; Stelkens et al., 2012; Linløkken et al., 2014). Understanding the impacts of barriers on brown trout demographic and genetic patterns at a river network scale is essential to advance our understanding on how river ecosystems are structured (Tonkin et al., 2018) and also to implement appropriate and efficient management and conservation strategies. However, to our knowledge no detailed empirical research exists investigating the responses of native brown trout populations to habitat fragmentation over a whole river network (from headwaters to the river mouth) considering its geometry and longitudinal connectivity (natural and anthropogenic barriers).

The present study aims at investigating the genetic consequences of altered connectivity on the brown trout population in the Deva-Cares river network and describing the patterns of population structure. We investigate the following: i) the genetic variability of brown trout in the catchment, (ii) the genetic differentiation and population structure, (iii) the historical migration rates and the effective population size and (iv) the causes of genetic differentiation and landscape characteristics. We hypothesize that changes in connectivity by longitudinal impermeable barriers will reduce gene flow and genetic variability producing isolation and increasing genetic differentiation in populations above barriers. Consequently, we hypothesize that isolated populations could have small

effective sizes. We will also consider different conservation and management strategies for the brown trout population in the Deva-Cares catchment based on the obtained results.

6.2 Methods

6.2.1 Fish survey

Between August and October 2014 (low-flow conditions and before the spawning period in the study area), a total of 197 brown trout of multiple year classes were collected by electrofishing from 13 locations from the main stream and tributaries of the Deva-Cares catchment (Figure 6.1). The study area includes several longitudinal barriers both anthropogenic and natural but other major significant pressures (Figure 6.1), such as stream habitat alteration, hydrological regime alteration or exotic species, are absent. This situation makes this catchment a suitable study area to investigate the genetic consequences of fragmentation and alteration of connectivity. The selection of these locations was done to cover the whole river network (from headwaters to the river mouth) and reaches above and below permeable and impermeable barriers (both natural and anthropogenic). Each survey was carried out on a minimum area that was representative of the river reach. Fish were sampled using a portable electric fishing device (power 1.3 kW), with direct current generation of 300 to 500 V, or pulsating up to 940 V with variable frequency (25 to 100 Hz). Natural barriers or nets were used to block the river reach in upstream and downstream directions in each field site. Individuals were sedated with eugenol and the adipose fin was removed and preserved in individually labelled tubes with 96% ethanol and stored at -16ºC for subsequent DNA analysis. Fish were placed into holding boxes till they recovered and then they were returned alive to the stream near the point of capture.

The river network that connects all study sites includes 29 longitudinal barriers (see Figure 6.1) of which only 6 are natural. Permeable and impermeable barriers were defined according to their characteristics, following a national standard for evaluating fish pass permeability (MAGRAMA, 2015). Permeable barriers were considered obstacles with a maximum height of jump ≤ 1m, minimum depth of the pool ≥ 1.25m height of jump, and maximum width crest ≤ 0.5m. Any other obstacle (e.g. with fish pass) was considered permeable, unless it had a non-functional fish pass. Moreover, all

obstacles present in the zone with presence of *Salmo salar* where considered also permeable for brown trout.

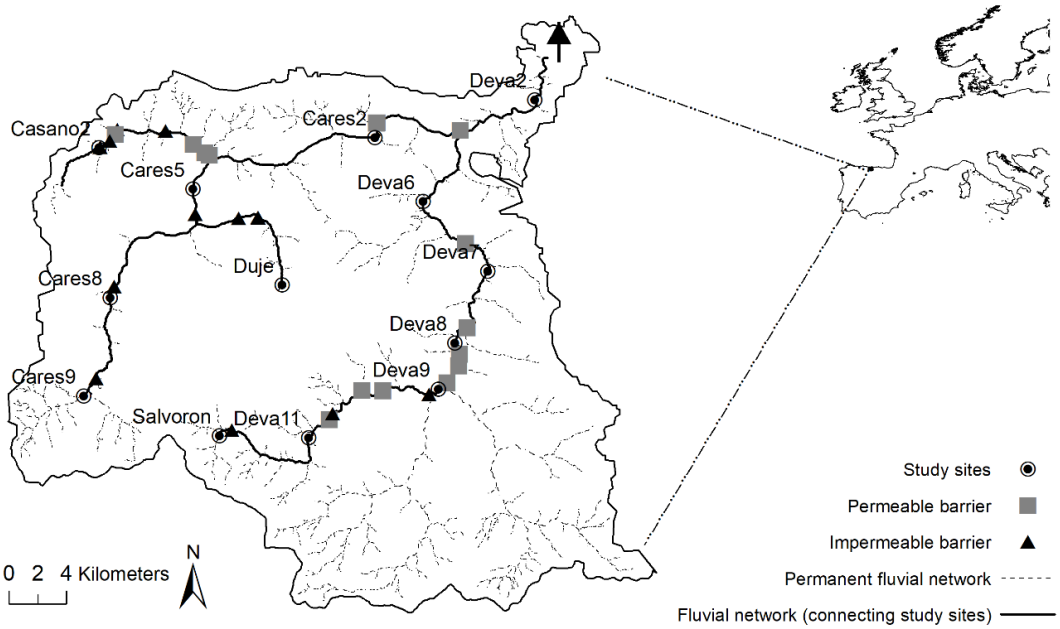

Figure 6.1. Location of surveyed river reaches between August and October 2014 and longitudinal barriers in the Deva-Cares catchment, Northern Spain.

6.2.2 River network and spatial data

The river network of the study area was obtained following a Virtual Watershed Approach (Barquin et al., 2015). The river network (divided in reaches; mean length of 500 m) is a digital representation of the surface water drainage network derived from a 25-m DEM (see González-Ferreras & Barquín, 2017 and Chapter III for more information about the delineation of the river network) that incorporates all the spatial information used in the riverscape genetic analysis. This spatial information is related to sample sites, barriers and topographical variables as slope or elevation. All the hydrological distances obtained between sample sites and barriers and differences in slope and elevation were calculated using ArcGIS Desktop 10.2 and ESRI`s ArcPy Python module (ESRI, 2014).

6.2.3 Molecular analyses

Total DNA was extracted from adipose fins using DNeasy Tissue Kit (QIAGEN, IZASA, Spain) and then stored at -20ºC. The quality and concentration of DNA was determined by spectrophotometry and was verified by 0.8% agarose gel electrophoresis.

12 microsatellite loci were amplified in three separate multiplex reactions (Supplementary material 6 Table S6.1) with forward primers labelled with a fluorescent dye. For each multiplex reaction, PCR multiplex was carried out in a final volume of 20 µl containing 10 µl of DNA AmpliTools Multiplex Master Mix (Biotools, Spain), 0.15-0.30 µM of each primer and 100ng genomic DNA template. Amplification reactions were performed using the following conditions: 95 ºC 15 min, 30 cycles of 95 ºC 30s, 58 ºC 1 min and 30 s and 72 ºC 1 min, with a final extension at 60 ºC for 30 min. Amplified PCR products were separated and visualized on an ABI Prism 3730 sequencer (Applied Biosystems, USA) and allele scoring was determined manually using PEAKSCANNER v1.0 (Applied Biosystems, USA).

6.2.4 Genetic analyses

Frequency of null alleles and scoring errors due to stuttering or large allelic drop-out were assessed using MICRO-CHECKER v2.2.3 (Van Oosterhout et al., 2004). The combined use of two or three methods is the best strategy for minimizing the false-positive and false-negative rates (Dąbrowski et al., 2014). For this reason, to test for the presence of null alleles we used two different methods in addition: CERVUS v3.0.3 (Kalinowski et al., 2007) and ML-NullFreq (Kalinowski & Taper, 2006).

Genetic variability within locations was estimated by the number of alleles across loci (A), observed (H_o) and expected (H_e) heterozygosity using GENETIX v4.05.2 (Belkhir et al., 2004), and allelic richness (A_R) was calculated with FSTAT v2.9.3 (Goudet, 1995). Tests for Hardy-Weinberg equilibrium and linkage disequilibrium were estimated for each locus and location with software GENEPOP v4.1 (Rousset, 2008). The significance was evaluated using Bonferroni corrections. Estimation of Wright's fixation indices for samples deviation from Hardy-Weinberg expectations for heterozygote disequilibrium (F_{IS}) were estimated following Weir and Cockerham (1984) using GENETIX v4.05.2 software. H_o, H_e, A and A_R values for all loci within each population were correlated with hydrologic distance to the most downstream location in this study (Deva2; Figure 6.1) using STATISTICA 8.0 (StatSoft Inc., USA).

Genetic differentiation (F_{ST}) among pair of populations was estimated in FSTAT v2.9.3 with sequential Bonferroni-corrected *p-values* after 10,000 random permutations. Population structure was further explored using the Bayesian clustering method

implemented in STRUCTURE v.2.3.4 (Pritchard et al., 2000). This method provides an unbiased estimate of population structure without prior information of the population affiliation of each individual (Stelkens et al., 2012). Structure analysis was running for 1 to 10 clusters (*K*) with ten replicates for each simulated cluster. Admixture model with correlated allele frequencies, 1,000,000 MCMC sweeps and a burn-in period of 2,500,000 steps were applying. Number of *K* was determined using STRUCTURE HARVESTER v0.6.94 (Evanno et al., 2005; Earl & vonHoldt, 2012). Replicates were aggregated using CLUMP 1.1.2 (Jakobsson & Rosenberg, 2007) and graphically displayed using DISTRUCT 1.1 (N. A. Rosenberg, 2004).

To investigate if the genetic differentiation among populations reflected contemporary patterns of stream connectivity, we used STREAMTREE software (Kalinowski et al., 2008). The relative genetic distances for each stream section among locations were estimated from the matrix of pairwise F_{ST} values. Fit of the STREAMTREE model to this matrix was quantified using a coefficient of determination (R^2).

Evidence for demographic bottlenecks was examined using two approaches. First, we tested for heterozygosity excess using BOTTLENECK v1.2.02 software (Cornuet & Luikart, 1996; S Piry et al., 1999), assuming a two-phase mutation model (TPM) with 80% stepwise mutations (SMM) (Di Rienzo et al., 1994) and 10,000 iterations. Significance of heterozygous excess was statistically tested by one-tailed Wilcoxon's signed-rank test. The BOTTLENECK software should be capable of detecting a population size reduction if the bottleneck was more recent, less severe and with small Θ value ($\Theta = 4N_e\mu$) after initial population decline (Williamson-Natesan, 2005). In addition, we estimated the ratio of the number of alleles to the range in allele size (*M-ratio*) described by Garza & Williamson (2001). *M*-ratio is most likely to correctly detect a population size reduction if the bottleneck was more ancient, prolonged and with large Θ value after initial population decline (Williamson-Natesan, 2005). *M-ratio* was estimated using M_P_VAL software (Garza & Williamson, 2001) and compared to a critical value of M (Mc) from theoretical population in mutation-drift equilibrium, implemented in CRITICAL_M software (Garza & Williamson, 2001) assuming pre-bottleneck effective population size of 50, 100, 500 and 1000 and a mutation rate (μ) of 5×10^{-4}. The proportion of one-step mutations (p_g) was set to 0.22 and the mean size of non one-step mutations (Δ_g) to 3.1, according to recommendations of Peery *et al*. (2012).

6.2.5 Migration and effective population size

Historical migration rates between the clusters defined by STRUCTURE were explored using coalescent method in MIGRATE-N 3.2.7 (Beerli, 2006; Beerli & Palczewski, 2010). Estimations of mutation-scaled migration rates M ($M = m/\mu$) and Θ ($\Theta = 4N_e\mu$) were calculated using a Brownian motion microsatellite model and Bayesian search strategy. Parameter space was searched using 10 short chains and one long chain with three replicates for 20,000,000 generations, an increment step of 20 and burn-in of 250,000. Parameter space was searched using four chains with an adaptive heating scheme (temperatures: 1.0, 1.2, 1.5, 3.0) to ensure that run results do not reflect local likelihood peaks.

Effective number of breeders over a reproductive year (N_b) was calculated for each location without migrants using the linkage disequilibrium (LD) method (Waples & Do, 2008) implemented in NeEstimator v.2.1 (Do et al., 2014). A minimum allele frequency cut-off value of 0.01 was employed and 95% confidence intervals were obtained using the jack-knife method. Migrants for each location were identified in GENECLASS2 (S. Piry et al., 2004) and removed because the LD method is constrained on the assumption of closed population model (Waples & Do, 2008). Bayesian statistical approach (Rannala & Mountain, 1997) with the Monte Carlo resampling method (Paetkau et al., 2004) of 1,000 simulated individuals and an alpha of 0.01 were used to identify individuals as migrants. The LD method showed consistent values across different demographic scenarios (Gilbert & Whitlock, 2015) and has been shown to be the best method to estimate the effective population size in populations with both low to no migration, small effective population sizes (Gilbert & Whitlock, 2015), and missing data adjustment (Peel et al., 2013). Considering a situation of overlapping generation, N_b estimates can be biased. Despite this, we applied the method developed by Waples *et al*. (2014) using two simple life history traits to adjust genetic estimates of N_b for correcting biases due to age structure. This N_b was adjusted using the ratio between adult life span (AL), age at maturity (α) and a coefficient of variation of age-specific fecundity (CVf), following the equation:

$$N_{b(adj)} = N_b / (0.991-0.206 \times \mathrm{Log}(AL)+0.256 \times \mathrm{Log}(\alpha)+0.137 CVf)$$

N_b can be more easily quantifiable, but remains less used than the effective population size over a generation (N_e) (Waples & Do, 2008). An average age at maturity of 2 years

was assumed for the river Deva-Cares. AL value was calculated as described by Waples *et al*. (2014) using a maximum breeding age (ω) of 5 years for Brown trout (personal communication from the Cantabria Regional Government). CVf was computed over recruitment data for both sexes with an estimated value of 0.196. $N_{e(adj)}$ was calculated using the equation proposed by Waples *et al.* (2014):

$N_{e(adj)} = N_{b(adj)} /(0.833+0.637xLog(AL)-0.793xLog(\alpha)-0.423xCVf)$

The effective size ratios $N_{b(adj)}/N_c$ and $N_{e(adj)}/N_c$ (Waples, 2006) were also calculated following Ferchaud *et al.* (2016) and Perrier *et al.* (2016). Estimates of census size (N_c) were calculated directly from the adult individuals captured in the first electrofishing pass. N_c was estimated from the sampling area (m^2) assuming a constant mean width in a length of 1 km.

6.2.6 Causes of genetic differentiation and riverscape characteristics

Testing for relationships between genetic differentiation and riverscape characteristics were examined using two approaches. First, Mantel test was used to test for the significance of correlation between linearized F_{STS} ($F_{ST}/(1-F_{ST})$) (Rousset, 1997) and location pairwise hydrological distance, difference in slope, elevation and number of total, permeable and impermeable barriers. Mantel tests with 9,999 permutations were conducted in the R v.3.3.3. package *ade4* v.1.7-11 (Thioulouse et al., 1997; Chessel et al., 2004). Decomposed pairwise regression analysis was used after each Mantel test to identify and remove potential outlier populations, which could be masking the effects of the tested riverscape variable (Koizumi et al., 2006). Afterwards, partial Mantel tests of significant variables were estimated in the *ade4* package in R.

Spatial analysis applications of the Mantel test in landscape genetics has been recently debated, rising concerns about its low statistical power and high type I error rates (P. Legendre & Fortin, 2010; Diniz-Filho et al., 2013; Guillot & Rousset, 2013; Pierre Legendre et al., 2015). To compare its performance with an alternative method, correlation between genetic differentiation and riverscape variables were tested using distance-based canonical redundancy analysis (dbRDA) of pairwise differentiation, implemented in the R package vegan v.2.4-6 (Oksanen et al., 2013). Riverscape variables were tested after transforming from a Euclidean matrix to continuous rectangular vector by principal coordinates analyses (PCoA). Significance of the

predictors was assessed using multivariate F-statistics. We first analyzed the relationship between linearized F_{ST} and each variable separately. Finally, we performed a partial dbRDA for each riverscape variable, controlling for the influence of hydrological distance (fitted as covariate).

6.3 Results

6.3.1 Genetic variability

11 microsatellite loci were analyzed, with an average ranging from 90% to 100% successful amplification depending on the locus. The locus *Sssp1605* proved difficult to amplify, with no allelic information in more than 14% of the individuals. Moreover, this locus showed reduced peaks and/or extra peaks of nonspecific binding or contamination origin. Thence, the locus *Sssp1605* was excluded from further analyses.

Results from MICRO-CHECKER, CERVUS and ML-NullFreq analysis allowed the possible occurrence of null alleles at one of the loci. However, there was no evidence for scoring errors due to stuttering or large drop-out. MICRO-CHECKER software exhibited evidence of null alleles for *SSOSL311* in most of the analyzed localities. The results from CERVUS ($F_{(null)} \geq 0.340$) and ML-NullFreq ($F_{(null)} \geq 0.200$, $p < 0.050$) showed similar results in this locus. Thus, the null alleles observed justified the elimination of this locus from the data analysis.

The genetic variability found was high (Table 6.1). All 10 out of 12 loci successfully analyzed were polymorphic, with a total of 120 alleles detected. Sampling localities exhibited on average a number of alleles ranging from 1.900 (Duje) to 6.600 (Deva9), average allelic richness between 1.859 (Duje) and 6.235 (Deva9) and an average expected and observed heterozygosity between 0.224-0.708 and 0.222-0.71, respectively. No evidence of linkage among pairs of loci was observed. Significant deviation from Hardy-Weinberg equilibrium was observed in 17% of the loci and in 31% of the localities (Table 6.1). However, only two loci: *SSOSL417* in Cares8 and BFRO002 in Deva8, remained significant after Bonferroni corrections. The loci SSOSL417 deviated from Hardy-Weinberg due to deficiency of heterozygotes, as indicated by high F_{IS} values (Table 6.1). The loci BFRO002 deviated from Hardy-Weinberg due to excess of heterozygotes, as indicated by negative F_{IS} values (Table 6.1).

Finally, a total of 17 private alleles were observed in 7 of the 13 populations (see Table 6.1).

Table 6.1. Genetic diversity indices for the Deva-Cares catchment: sample size (*N*), observed number of alleles (*A*), allelic richness (A_R), private alleles (*Pa*), expected (H_e) and observed heterozygosity (H_o), F_{IS} values and deviations from Hardy-Weinberg equilibrium (*HWE*). Significant values following the Bonferroni correction are indicated by an asterisk (*).

			Locus									
		Total	***Str15***	***Str60***	***Str73***	***Ssa85***	***Ssa197***	***SSOSL85***	***SS4***	***SSOSL417***	***SSOSL438***	***BFRO002***
Cares9	*N*	15	15	15	15	15	15	15	14	12	15	15
	A	5.500	4	5	2	6	8	7	6	7	6	4
	A_R	5.250	3.800	4.599	2.000	5.599	7.559	6.361	5.825	7.000	5.790	3.965
	Pa	1	0	1	0	0	0	0	0	0	0	0
	H_o	0.629	0.467	0.733	0.533	0.733	0.667	0.667	0.643	0.583	0.733	0.533
	H_e	0.683	0.651	0.580	0.480	0.760	0.822	0.658	0.686	0.788	0.778	0.629
	F_{IS}	0.115	0.315	-0.232	-0.077	0.070	0.222	0.021	0.100	0.300	0.091	0.186
	HWE	0.009	0.039	0.144	0.596	0.421	0.081	0.460	0.039	0.024	0.334	0.086
Cares8	*N*	15	15	15	15	15	15	15	13	14	14	15
	A	5.600	4	6	3	6	9	6	6	5	7	4
	A_R	5.426	3.999	5.956	2.966	5.994	8.360	5.595	5.769	4.982	6.838	3.799
	Pa	3	0	0	0	0	0	1	1	1	0	0
	H_o	0.660	0.667	1.000	0.400	0.933	0.867	0.600	0.539	0.286	0.643	0.667
	H_e	0.708	0.676	0.782	0.540	0.820	0.838	0.733	0.580	0.694	0.793	0.620
	F_{IS}	0.103	0.048	-0.246	0.291	-0.104	0.000	0.215	0.111	0.612	0.225	-0.041
	HWE	0.011	0.272	0.031	0.161	0.270	0.369	0.038	0.293	0.001*	0.014	0.393
Duje	*N*	16	16	16	16	16	16	16	16	13	15	16
	A	1.900	1	2	1	3	2	1	1	3	3	2
	A_R	1.859	1.000	2.000	1.000	2.996	2.000	1.000	1.000	2.997	2.800	1.800
	Pa	0	0	0	0	0	0	0	0	0	0	0
	H_o	0.222	0.000	0.438	0.000	0.500	0.500	0.000	0.000	0.385	0.333	0.067
	H_e	0.224	0.000	0.342	0.000	0.406	0.375	0.000	0.000	0.565	0.487	0.064
	F_{IS}	0.043	-	-0.250	-	-0.200	-0.304	-	-	0.355	0.346	-
	HWE	0.338	-	0.435	-	0.307	0.314	-	-	0.018	0.125	-
Cares5	*N*	14	13	14	12	13	12	13	12	14	14	12
	A	5.000	5	4	5	4	4	8	3	8	6	3
	A_R	4.897	4.917	3.984	5.000	3.920	4.000	7.769	3.000	7.665	5.713	3.000
	Pa	0	0	0	0	0	0	0	0	0	0	0
	H_o	0.611	0.462	0.500	0.500	0.462	0.750	0.846	0.417	0.714	0.714	0.750
	H_e	0.638	0.624	0.602	0.611	0.476	0.677	0.825	0.559	0.755	0.707	0.538
	F_{IS}	0.081	0.298	0.205	0.224	0.071	-0.065	0.015	0.295	0.091	0.026	-0.356
	HWE	0.064	0.012	0.024	0.029	0.390	0.502	0.538	0.026	0.052	0.257	0.088

Table 6.1. (Continued)

			Locus									
		Total	***Str15***	***Str60***	***Str73***	***Ssa85***	***Ssa197***	***SSOSL85***	***SS4***	***SSOSL417***	***SSOSL438***	***BFRO002***
Casano2	*N*	15	15	15	15	15	14	14	14	13	15	15
	A	2.900	3	4	3	4	2	4	1	4	2	2
	A_R	2.798	2.995	3.799	2.766	3.795	2.000	3.714	1.000	3.920	1.995	2.000
	Pa	0	0	0	0	0	0	0	0	0	0	0
	H_o	0.400	0.467	0.600	0.200	0.667	0.500	0.500	0.000	0.462	0.200	0.400
	H_e	0.384	0.571	0.540	0.184	0.533	0.436	0.564	0.000	0.512	0.180	0.320
	F_{IS}	-0.004	0.216	-0.077	-0.050	-0.217	-0.110	0.150	-	0.138	-0.077	-0.217
	HWE	0.496	0.256	0.508	0.897	0.203	0.592	0.368	-	0.353	0.899	0.540
Cares2	*N*	15	15	15	15	15	15	15	14	14	14	14
	A	5.200	3	4	5	5	6	8	5	8	5	3
	A_R	4.893	2.800	3.790	4.595	4.561	5.785	7.556	4.571	7.270	4.997	3.000
	Pa	0	0	0	0	0	0	0	0	0	0	0
	H_o	0.591	0.533	0.400	0.467	0.333	0.733	0.867	0.500	0.857	0.643	0.571
	H_e	0.593	0.504	0.391	0.627	0.396	0.691	0.807	0.546	0.656	0.686	0.630
	F_{IS}	0.040	-0.023	0.012	0.287	0.191	-0.027	-0.040	0.121	-0.274	0.100	0.130
	HWE	0.231	0.611	0.579	0.016	0.215	0.562	0.359	0.261	0.041	0.365	0.396
Salvoron	*N*	15.000	15	15	15	15	15	15	15	13	13	13
	A	2.100	2	2	1	1	3	2	2	4	2	2
	A_R	2.065	2.000	1.800	1.000	1.000	2.966	1.966	2.000	3.920	2.000	1.997
	Pa	0	0	0	0	0	0	0	0	0	0	0
	H_o	0.249	0.533	0.067	0.000	0.000	0.400	0.133	0.200	0.615	0.385	0.154
	H_e	0.241	0.498	0.064	0.000	0.000	0.480	0.124	0.278	0.512	0.311	0.142
	F_{IS}	0.004	-0.037	0.000	-	-	0.200	-0.037	0.312	-0.164	-0.200	-0.044
	HWE	0.482	0.648	-	-	-	0.244	0.967	0.327	0.326	0.629	0.960
Deva11	*N*	15.000	15	15	15	15	15	15	15	15	15	15
	A	5.100	6	4	3	4	6	5	7	8	5	3
	A_R	4.779	5.400	3.930	2.961	3.765	5.361	4.921	6.760	7.195	4.566	2.931
	Pa	2	1	0	0	0	1	0	0	0	0	0
	H_o	0.493	0.533	0.400	0.333	0.333	0.333	0.533	0.733	0.933	0.533	0.267
	H_e	0.519	0.644	0.436	0.291	0.389	0.444	0.527	0.816	0.789	0.616	0.240
	F_{IS}	0.084	0.206	0.116	-0.111	0.177	0.282	0.022	0.135	-0.150	0.167	-0.077
	HWE	0.053	0.244	0.075	0.673	0.226	0.138	0.078	0.093	0.178	0.279	0.791
Deva9	*N*	15.000	15	15	15	15	15	15	15	15	14	14
	A	6.600	6	3	6	5	10	11	7	9	6	3
	A_R	6.235	5.955	2.966	5.531	4.897	9.316	10.257	6.565	8.155	5.712	3.000
	Pa	3	0	0	0	0	1	0	1	1	0	0
	H_o	0.710	0.933	0.400	0.600	0.600	0.933	0.800	0.733	0.667	0.643	0.786
	H_e	0.689	0.769	0.451	0.589	0.558	0.829	0.873	0.773	0.736	0.679	0.630
	F_{IS}	0.005	-0.181	0.147	0.016	-0.041	-0.092	0.118	0.086	0.128	0.090	-0.212
	HWE	0.460	0.098	0.347	0.440	0.894	0.277	0.045	0.031	0.143	0.421	0.157

Table 6.1. (Continued)

			Locus									
		Total	***Str15***	***Str60***	***Str73***	***Ssa85***	***Ssa197***	***SSOSL85***	***SS4***	***SSOSL417***	***SSOSL438***	***BFRO002***
Deva8	*N*	15.000	15	15	15	15	15	15	14	13	13	13
	A	6.400	6	5	5	3	9	10	7	8	8	3
	A_R	6.074	5.925	4.955	4.765	2.800	7.964	9.125	6.823	7.615	7.766	3.000
	Pa	0	0	0	0	0	0	0	0	0	0	0
	H_o	0.692	0.733	0.733	0.467	0.267	0.733	0.800	0.643	0.615	0.923	1.000
	H_e	0.669	0.696	0.598	0.656	0.331	0.704	0.807	0.750	0.692	0.796	0.660
	F_{IS}	0.003	-0.020	-0.194	0.319	0.228	-0.007	0.043	0.179	0.150	-0.121	-0.486
	HWE	0.462	0.428	0.172	0.010	0.335	0.452	0.445	0.043	0.230	0.520	0.007*
Deva7	*N*	17.000	17	17	17	17	17	17	17	17	16	17
	A	6.500	4	5	2	5	7	10	8	12	8	4
	A_R	5.738	3.900	4.680	2.000	4.680	6.016	8.856	6.803	9.756	7.000	3.686
	Pa	3	0	0	0	0	0	0	1	2	0	0
	H_o	0.610	0.529	0.471	0.471	0.647	0.529	0.882	0.706	0.765	0.625	0.471
	H_e	0.647	0.552	0.574	0.498	0.574	0.583	0.822	0.763	0.744	0.797	0.559
	F_{IS}	0.088	0.071	0.210	0.086	-0.097	0.122	-0.044	0.105	0.002	0.246	0.187
	HWE	0.020	0.278	0.235	0.561	0.545	0.265	0.528	0.009	0.706	0.029	0.182
Deva6	*N*	15.000	14	15	14	15	15	15	14	15	15	14
	A	5.800	5	3	6	4	7	8	5	10	7	3
	A_R	5.444	4.714	2.990	5.825	3.795	6.365	7.161	4.967	8.897	6.721	3.000
	Pa	3	0	0	1	0	1	0	0	1	0	0
	H_o	0.653	0.857	0.400	0.714	0.400	0.667	0.733	0.643	0.733	0.600	0.786
	H_e	0.628	0.653	0.340	0.709	0.433	0.707	0.736	0.589	0.780	0.702	0.630
	F_{IS}	-0.005	-0.279	-0.143	0.030	0.111	0.091	0.038	-0.054	0.094	0.179	-0.212
	HWE	0.462	0.073	0.549	0.058	0.107	0.565	0.047	0.760	0.055	0.065	0.161
Deva2	*N*	15.000	15	15	15	15	15	15	14	15	15	15
	A	6.300	7	3	5	6	9	9	7	9	5	3
	A_R	5.923	6.531	2.931	4.766	5.599	8.457	7.995	6.712	8.521	4.726	2.995
	Pa	2	1	0	0	0	0	0	1	0	0	0
	H_o	0.611	0.800	0.267	0.600	0.800	0.667	0.667	0.643	0.733	0.333	0.600
	H_e	0.664	0.760	0.240	0.722	0.740	0.782	0.791	0.750	0.840	0.442	0.571
	F_{IS}	0.114	-0.018	-0.077	0.203	-0.047	0.181	0.191	0.179	0.161	0.278	-0.016
	HWE	0.005	0.513	0.785	0.123	0.472	0.157	0.053	0.019	0.060	0.177	0.549

6.3.2 Genetic differentiation

Correlations between *A*, A_R, H_o and H_e with distance to the most downstream location (Deva2) were observed only for *A* ($p = 0.048$, see Table 6.2 and Figure 6.2). In the case of H_e and A_R the values were marginally significant ($p < 0.100$) while for H_o correlations were not observed.

Table 6.2. Correlations between A, A_R, H_o and H_e with distance to the most downstream location (Deva2). Significant values are indicated by an asterisk (*).

	Mean	**r(X,Y)**	**r²**	**t**	**p**	**Constant**	**Slope**
A	4,992	-0,558	0,311	-2,230	0,048*	6,819	-0,000057
Ar	4,713	-0,548	0,300	-2,172	0,053	6,350	-0,000051
Ho	0,553	-0,450	0,202	-1,669	0,123	0,700	-0,000005
He	0,556	-0,508	0,258	-1,954	0,077	0,725	-0,000005

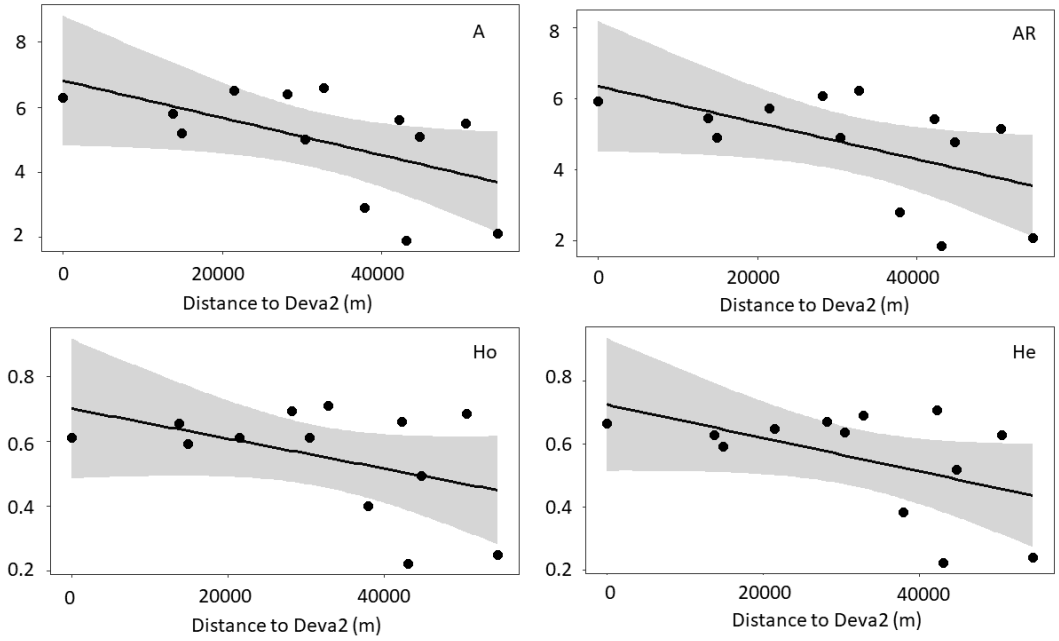

Figure 6.2. Linear regression between A, A_R, H_o and H_e with distance to the most downstream location (Deva2) and 95% confidence intervals (results are represented in Table 6.2).

Overall genetic population differentiation (F_{ST} = 0.181) was great (Hartl et al., 1997), yielding a range of F_{ST} estimates from 0.002 (Deva8 and Cares2-Deva6) to 0.664 (Salvoron-Duje; Table 6.3). 65 of the 78 pairwise population comparisons were significant after Bonferroni correction (Table 6.3). Duje, Casano2, and Salvoron were markedly different from each other and had genetic distinctiveness in comparison to the rest of localities (F_{ST} = 0.572). When comparing the F_{ST} values in those three genetic units with the rest of localities, they were very great for Duje (F_{ST} = 0.261), moderate for Casano2 (F_{ST} = 0.106) and great for Salvoron (F_{ST} = 0.176). The rest of localities showed a moderate F_{ST} mean value (F_{ST} = 0.079). Some localities pairs exhibited greater genetic dissimilarity than others, ranging from 0.002 (Deva8 and Cares2-Deva6) to 0.198 (Deva11-Cares9).

Table 6.3. Pairwise F_{ST} values for the river Deva-Cares (below diagonal). Associated *p-values* are shown above diagonal. Significant values following the Bonferroni correction are indicated by an asterisk (*).

	Cares9	**Cares8**	**Duje**	**Cares5**	**Casano2**	**Cares2**	**Salvoron**	**Deva11**	**Deva9**	**Deva8**	**Deva07**	**Deva06**	**Deva02**
Cares9		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Cares8	0.033		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Duje	0.419	0.384			0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Cares5	0.128	0.103	0.404		0.001*	0.011	0.001*	0.001*	0.051	0.001*	0.001*	0.001*	0.001*
Casano2	0.262	0.226	0.546	0.133		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Cares2	0.148	0.128	0.371	0.020	0.138		0.001*	0.001*	0.026	0.004	0.003	0.084	0.001*
Salvoron	0.372	0.376	0.664	0.346	0.508	0.350		0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
Deva11	0.198	0.188	0.455	0.109	0.228	0.107	0.145		0.001*	0.001*	0.001*	0.001*	0.001*
Deva09	0.115	0.111	0.354	0.018	0.177	0.014	0.291	0.073		0.019	0.046	0.039	0.008
Deva08	0.129	0.115	0.337	0.043	0.173	0.010	0.317	0.102	0.015		0.02	0.16	0.001*
Deva07	0.153	0.142	0.335	0.078	0.247	0.034	0.338	0.135	0.02	0.015		0.001*	0.001*
Deva06	0.145	0.129	0.364	0.038	0.145	0.002	0.312	0.084	0.014	0.002	0.038		0.001*
Deva02	0.118	0.119	0.412	0.049	0.155	0.034	0.342	0.112	0.026	0.042	0.069	0.03	

The results of the Evanno test suggested that $K = 5$ was the most probable cluster number for the STRUCTURE analysis (Figure 6.3). Populations were assigned to each one of the cluster based on a *Q value* > 0.500: 1) Cares9 and Cares8, 2) Duje, 3) Casano2, 4) Salvoron and 5) Cares5, Cares2, Deva9, Deva8, Deva7, Dava6 and Deva2. Deva11 exhibited high admixture between the fourth and fifth cluster, and was considered as an independent group between the fourth gene pool ($Q = 0.479$) and fifth ($Q = 0.329$) for subsequent analysis. All upstream localities were genetically different while most of the rest of the downstream localities were comparatively homogeneous. Cares8 had the highest *Q value* ($Q = 0.981$) and was included in the first cluster. Cares5 and Cares2 showed an admixture of genotypes between the third and fifth clusters. Those populations were assigned to fifth clusters due to *Q values* obtained ($Q \geq 0.577$).

Genetic distances for each stream section among locations were estimated by STREAMTREE (Figure 6.4). The coefficient of determination ($R^2 = 0.972$) evidenced a good fit to the observed data. The results were useful in visualizing and quantifying the resistance to migration in the river Deva-Cares. The STREAMTREE analysis was highest between Duje, Casano2 and Salvoron and the rest of downstream localities. Genetic distances at or close to zero were assigned to stream sections without barriers or with low density of permeable barriers (Figure 6.4).

The BOTTLENECK tests showed evidence for recent bottlenecks in Cares8 population (Table 6.4). Ancient bottleneck was detected in Deva6 using the M-ratio analysis by M-ratio value lower than its M-critical value (Table 6.4). Statistical significance to detect historical bottleneck was not maintained in this locality assuming Θ values of 0.1 to 2.

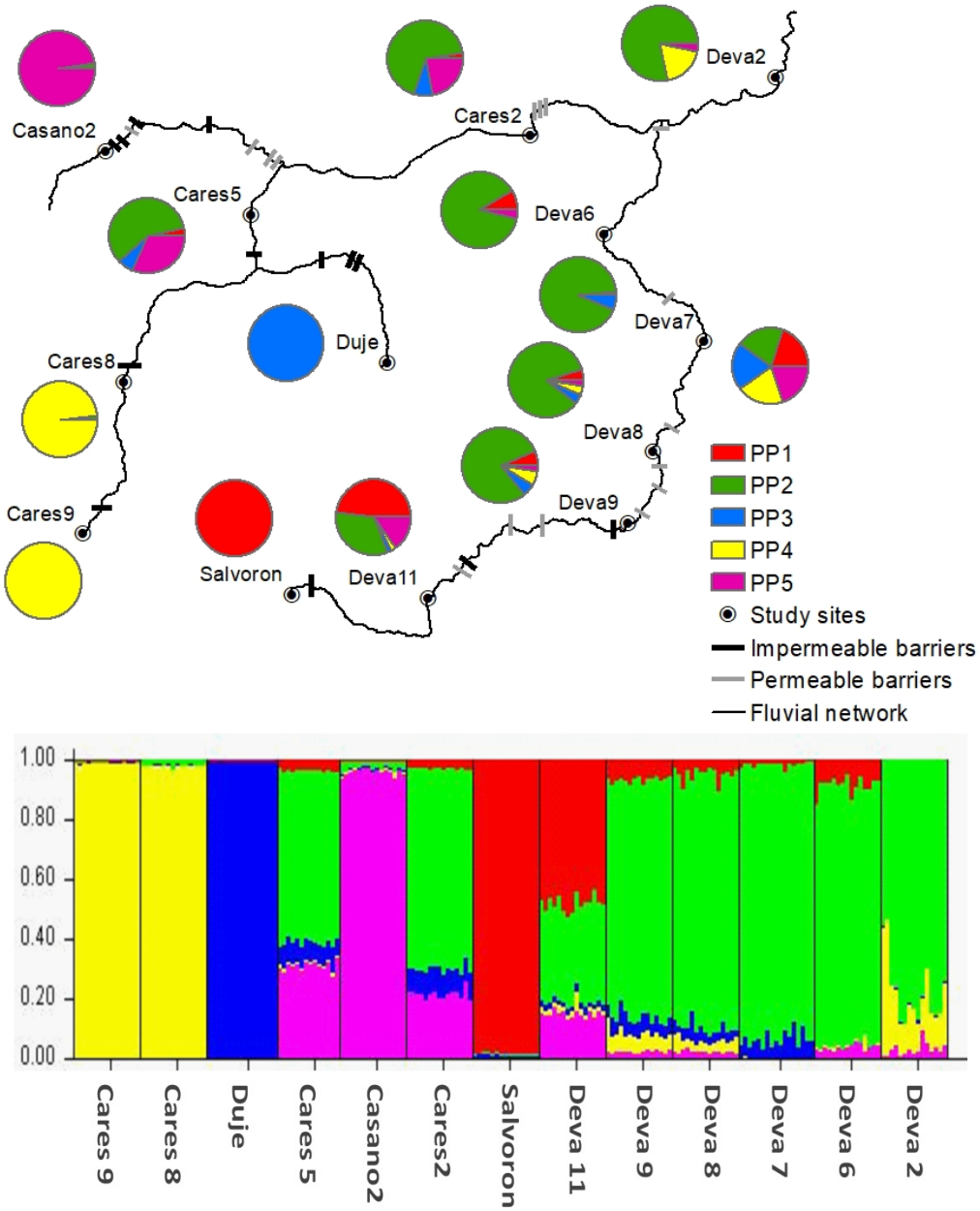

Figure 6.3. Geographical distribution of genetic clusters identified by STRUCTURE and clustering analysis results for K = 5. (PP1, PP2, PP3, PP4 and PP5 represent parental populations). Colored bars represent proportions of membership of each individual to each cluster.

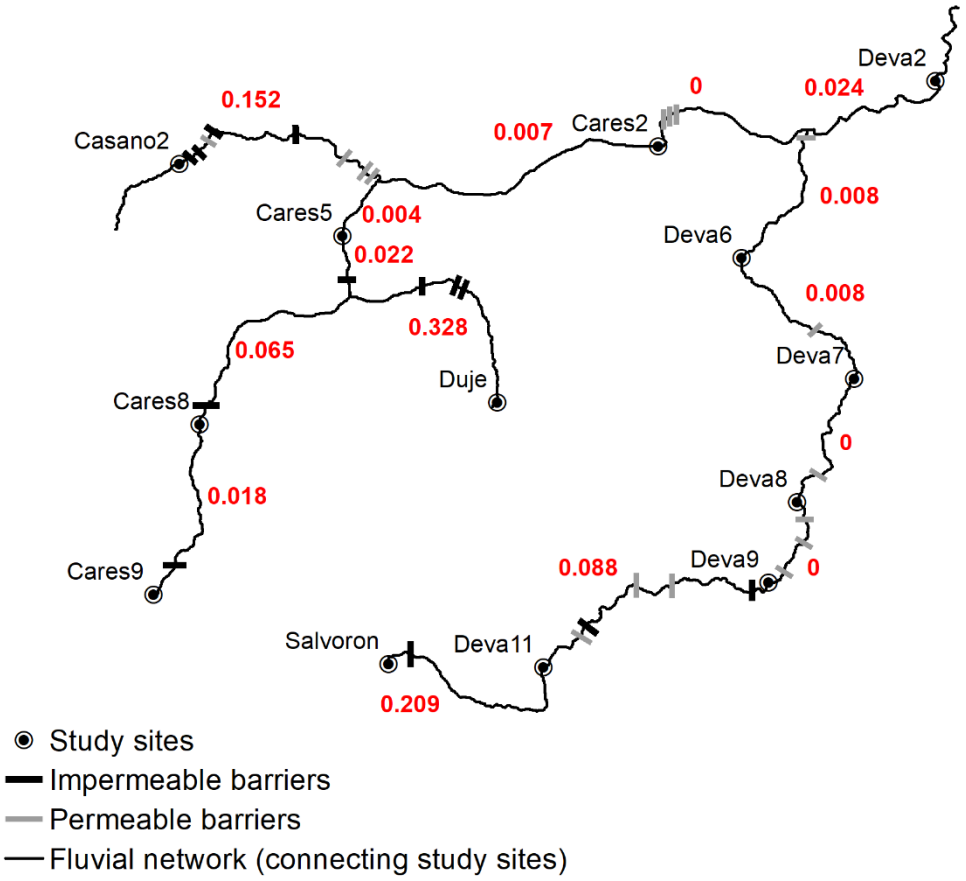

Figure 6.4. Genetic distances associated with stream sections calculated using STREAMTREE analysis.

Table 6.4. Bottleneck tests for the river Deva-Cares. Expected recent bottleneck is presented as *p-values* from Wilcoxon's signed-rank test, assuming a TPM model. *M-ratio* values below the critical threshold (M_c) are indicated in bold.

Sample	TPM	*M-ratio*	M_c			
			Θ = 0.1	**Θ = 0.2**	**Θ = 1.0**	**Θ = 2.0**
Cares9	p=0.278	1.289	0.770	0.758	0.699	0.666
Cares8	**p=0.042**	1.127	0.770	0.758	0.699	0.666
Duje	p=0.344	0.882	0.773	0.761	0.703	0.670
Cares5	p=0.577	0.775	0.770	0.760	0.701	0.664
Casano2	p=0.715	0.925	0.770	0.758	0.699	0.666
Cares2	p=0.903	0.961	0.770	0.758	0.699	0.666
Salvoron	p=0.727	1.030	0.770	0.758	0.699	0.666
Deva11	p=0.998	1.317	0.770	0.758	0.699	0.666
Deva9	p=0.754	1.100	0.770	0.758	0.699	0.666
Deva8	p=0.991	0.838	0.770	0.758	0.699	0.666
Deva7	p=0.984	1.192	0.773	0.758	0.704	0.667
Deva6	p=0.991	**0.757**	0.770	0.758	0.699	0.666
Deva2	p=0.784	1.334	0.770	0.758	0.699	0.666

6.3.3 Migration and effective population size

Migration rate estimates between groups and localities were low, ranged from 0.2 to 2.8% migrants/generation. All pairwise comparisons suggested symmetric gene flow according to non-overlapping 95% CIs (Figure 6.5).

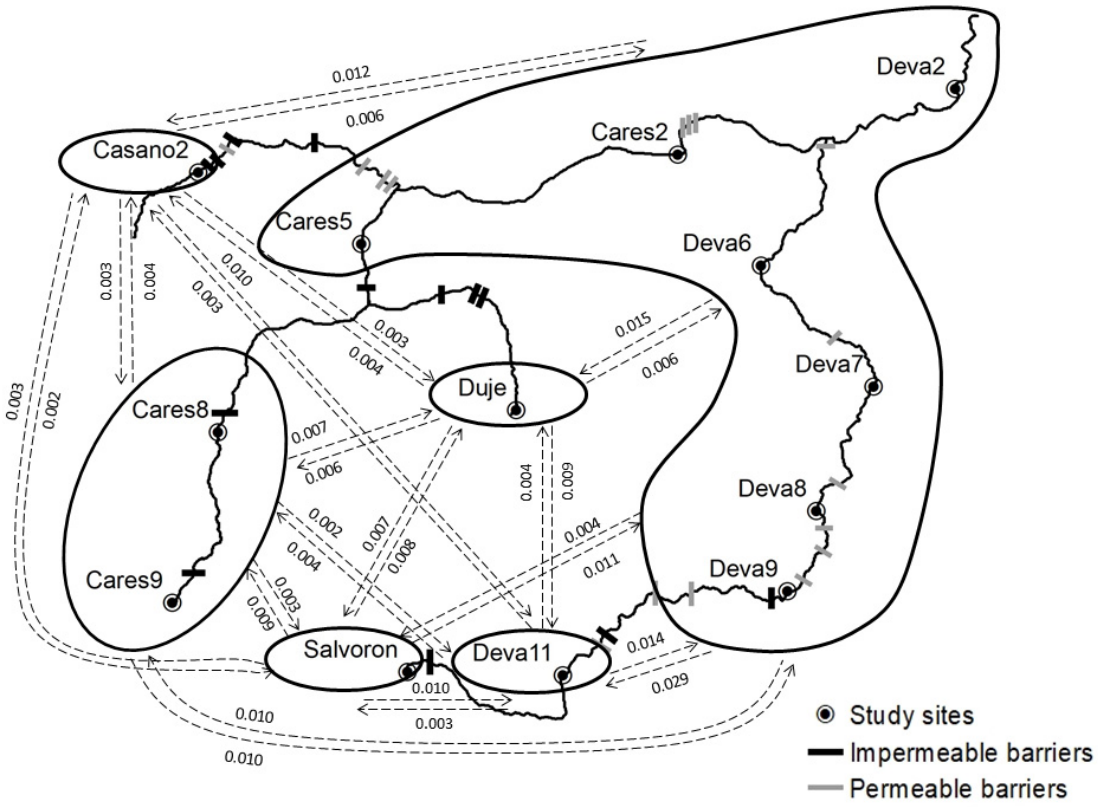

Figure 6.5. Graphical representation of migrations estimates in the Deva-Cares river using MIGRATE.

Most of the population exhibited low to moderate effective population sizes (Table 6.5). Estimates for Casano2 were infinite and was not included in subsequent analyses. Only Cares9, Cares2 and Deva6 showed $N_e > 50$, and only Cares2 exhibited $N_e > 100$. The effective number of breeders (N_b) estimated with LDNe were low or moderate, yielding a range of N_b estimates from 2 to 218 (mean = 48). $N_{b(adj)}$ estimates were higher than non-adjusted values and went up from 2 to 258 (mean = 57). The estimated ratio between adjusted effective number of breeders $N_{b(adj)}$ and census size (N_c) varied from an average of 0.124 in Deva2 to 4.865 in Cares2. The estimated ratio between adjusted effective size and census size $N_{e(adj)}/N_c$ changed from 0.099 in Duje to 3.809 in Cares2.

Table 6.5. Estimate of census size (N_c, [20-40%]), effective number of breeders (N_b, [CI 95%]), adjusted number of breeders ($N_{b(adj)}$), adjusted effective size ($N_{e(adj)}$) and the effective size ratios $N_{b(adj)}/N_c$ and $N_{e(adj)}/N_c$, from brown trout populations of river Deva-Cares.

Sample	N_c	*Migrants removed*	N_b [CI 95%]	$N_{b(adj)}$ [CI 95%]	$N_{e(adj)}$ [CI 95%]	$N_{b(adj)}/N_c$	$N_{e(adj)}/N_c$
Cares9	108	1	57 [16-∞]	67 [19-∞]	53 [15-∞]	0.623	0.488
Cares8	350	0	44 [14-∞]	52 [16-∞]	40 [13-∞]	0.147	0.115
Duje	15	0	2 [1-15]	2 [1-18]	2 [1-14]	0.126	0.099
Cares5	40	0	33 [11-∞]	39 [13-∞]	30 [10-∞]	0.968	0.758
Casano2	483	0	∞ [10-∞]	∞ [12-∞]	∞ [9-∞]	-	-
Cares02	53	1	218 [19-∞]	258 [22-∞]	202 [17-∞]	4.865	3.809
Salvoron	10	0	5 [1-∞]	6 [1-∞]	4 [1-∞]	0.567	0.444
Deva11	208	0	22 [9-512]	26 [11-604]	20 [8-473]	0.125	0.098
Deva9	127	0	33 [18-101]	38 [21-119]	30 [17-93]	0.302	0.237
Deva8	114	0	18 [9-66]	22 [11-78]	17 [8-61]	0.190	0.148
Deva7	75	2	30 [13-1001]	36 [15-1182]	28 [12-924]	0.477	0.373
Deva6	64	2	106 [22-∞]	126 [26-∞]	98 [21-∞]	1.961	1.535
Deva2	57	1	6 [3-11]	7 [3-13]	6 [3-10]	0.124	0.097

6.3.4 Causes of genetic differentiation

There was a significant pattern of isolation by distance-IBD (Mantel test; $r^2 = 0.232$, $p < 0.050$; Table 6.6). Moreover, the presence of total barriers and impermeable barriers showed a significant pattern of isolation by barriers (Mantel test; $r^2 = 0.322$, $p < 0.050$ and $r^2 = 0.700$, $p < 0.050$, respectively). On the contrary, elevation, slope and presence of permeable barriers were not significant. The dbRDA analysis confirmed the above findings by Mantel test. Results of dbRDA showed a significant pattern of isolation by distance and isolation by impermeable barriers ($r^2 = 0.951$, $p < 0.050$ and $r^2 = 0.640$, $p < 0.05$ respectively). Partial Mantel test showed a significant pattern of isolation by impermeable barriers after controlling for the effects of hydrological distance and total barriers (partial Mantel test; r^2=0.610, $p < 0.050$ and $r^2 = 0.558$, $p < 0.050$ respectively) showing that the influence of impermeable barriers is higher than hydrological distance and total barriers.

Table 6.6. Simple Mantel and partial Mantel test between F_{ST} and hydrological distance, total barriers, and impermeable barriers. Simple mantel test also contains elevation, slope and permeable barriers. Bold results are significant ($p < 0.050$).

	Mantel test	r^2	p
F_{ST}	Hydrological distance	**0.232**	**<0.001**
	Barriers	**0.322**	**0.006**
	Permeable barriers	0.096	0.063
	Impermeable barriers	**0.700**	**<0.001**
	Elevation	0.006	0.693
	Slope	0.009	0.250

	Partial Mantel test	Controlled by	r^2	p
F_{ST}	Hydrological distance	Barriers	0.001	0.483
	Barriers	Hydrological distance	0.118	0.108
	Hydrological distance	Impermeable barriers	0.004	0.316
	Impermeable barriers	Hydrological distance	**0.611**	**0.002**
	Barriers	Impermeable barriers	-0.002	0.566
	Impermeable barriers	Barriers	**0.556**	**0.001**

6.4 Discussion

The study revealed significant genetic differentiation among sites within the catchment and significant isolation effect by barriers. Our results are consistent with our initial hypothesis and they also agree other studies on showing the importance of longitudinal barriers in river networks on fish population structure (Yamamoto et al., 2004; Griffiths et al., 2009) and its possible effect associated to genetic drift, loss of genetic diversity or isolation (Yamamoto et al., 2004; Horreo et al., 2011; Stelkens et al., 2012).

6.4.1 Genetic diversity and population structuring

Microsatellite loci have been used in multiple studies as an efficient resource in the description of the genetic variability and population structure of fish populations within-watershed (McGlashan et al., 2001; Wofford et al., 2005; Griffiths et al., 2009) and among-watershed (Huey et al., 2010). Allelic richness presents a slightly broader range (1.859 to 6.235) than that obtained by Horreo *et al.* (2011) in a previous study in the Deva-Cares catchment (1.394 to 4.778). Meanwhile, observed and expected heterozygosity have a slightly narrower range (H_e = 0.224-0.708, H_o = 0.222-0.710)

than the previous study (H_e = 0.130-0.805, H_o = 0.154-0.742). The global population differentiation across all 13 samples sites (F_{ST} = 0.181) was considered as great (Hartl et al., 1997) as in the Horreo et al. (2011) results (F_{ST} = 0.204).

Genetic diversity is distributed heterogeneously, revealing the existence of several populations genetically differentiated. This indicates that drift and gene flow have interplayed in shaping the genetic constitution of the brown trout populations, as has been shown in other studies (e.g. Massa-Gallucci et al., 2010). In our study, below-barrier populations showed high levels of genetic diversity and lower F_{ST} values. By the contrary, headwater and above-barrier populations showed low levels of genetic diversity and high F_{ST} values, evidence that supports fast rates of genetic drift. In fact, these populations are the only ones presenting fixed alleles (Duje = 4, Casano2 = 1 and Salvoron = 2). This result has also been found for above-barrier populations in other studies of salmonids (Neville et al., 2009). Moreover, we found concordance between impermeable barriers and the identified genetic groups in STRUCTURE analysis, which together with the STREAMTREE results supports the role of impermeable barriers in shaping population structure, acting as an isolation mechanism.

All populations separated by one or more impermeable barrier belonged to different genetic groups, excepting Cares 8 and Cares 9. The barrier between both populations is the most recently built dam in the catchment (constructed in 1995). Quite likely, not enough time has passed yet to cause genetic divergence among these populations, because genetic differentiation is correlated with the time since physical isolation by barrier started (Yamamoto et al., 2004). However, F_{ST} values between both populations were significant, indicating that in the future both populations may diverge. A previous study with sample data from 2007 (Horreo et al., 2011) showed similar results in the Deva-Cares catchment separating this genetic unit from the rest of the catchment. However, our study included a more detailed representation of the barriers in the catchment and more samples above and below barriers showing more genetic units in the catchment than those previously identified.

Significant F_{ST} values among populations where dispersal is possible (by the presence of permeable barriers or absence of obstacles) indicate that minor genetic structure exists and allele frequencies differed throughout the permeable area. These differentiations could be explained by a certain degree of reduction of gene flow caused

by permeable barriers. In the case of populations where there is no barrier between localities (e.g. Cares2-Cares5), genetic differentiation could be due to effects not covered by this study. Previous studies have shown that environmental factors such as geological substrate (C. Perrier et al., 2011) or temperature (Dionne et al., 2008) may influence gene flow.

6.4.2 Gene flow and riverscape genetics: influence on genetic differentiation

Populations showed a significant IBD pattern, which is common in stream salmonids with movers and no movers (Kanno et al., 2011) and other fish species (Sotola et al., 2017). In addition, we report a decrease in genetic variability (number of alleles and allelic richness) in upstream sites and a downstream-biased gene flow. Similar tendencies have been found in other fish species (*Poecilia reticulata*; Barson et al., 2009; *Cotus gobio*; Junker et al., 2012; *Oncorhynchus mykiss*; Winans et al., 2015). Evidence of correlation between distance to most downstream location and number of alleles and allelic richness but not heterozygosity, could be explained because number of alleles and allelic richness approach equilibrium more rapidly than heterozygosity (Epps & Keyghobadi, 2015; Salisbury et al., 2016). Barson et al. (2009) reported that populations with downstream-biased gene flow may act as sink in lowland populations and as source in upland populations contributing gene flow into the downstream populations. Thus, in our study we have found characteristics of both metapopulation (i.e. downstream-biased gene flow) and member-vagrant models (i.e. significant effect of IBD). According to the study in *Salmo salar* realized by Garant, Dodson & Bernatchez (2000) both models are not mutually exclusive and their combined use may help better understanding the dynamics of the genetic structure in unstable environments. Thus, the member vagrant hypothesis generates the number of subpopulations given the life cycle and habitat structure of a species, while the metapopulation model explain the extent of genetic divergence among subpopulations and its temporal persistence. Both models were also found to explain the population genetic structure of the Japanese eel *Anguilla japonica* (Tseng et al., 2006). Nevertheless, it would be needed temporal studies to identify the presence or absence of temporal instability in order to identify in detail a particular evolutionary model in the Deva-Cares catchment.

Results from historical migration in our study indicate that gene flow between all pairwise comparisons is symmetric. Although a detailed analysis of recent migration in the Deva-Cares catchment would be needed (e.g. BAYESASS; Wilson & Rannala, 2003), the downstream-biased gene flow reported could be caused by the fragmentation. Previous studies have shown that in presence of barriers some individuals are transported downstream by the water flow and barriers amplify the dispersal asymmetry because of negligible upstream dispersal (Gomez-Uchida et al., 2009; Junker et al., 2012) while symmetric migration is suggested in absence of barriers (Gomez-Uchida et al., 2009; Horreo et al., 2011). In our study, reaches below the barriers contain a high proportion of mixed genetic pools that could be the result of immigrants from above (see Figure 6.3). In fact, isolation by barriers appeared to play a more important role than hydrological distance on the genetic structure of the populations in the studied catchment. This pattern has also been found in previous studies analyzing fragmentation in river systems (Meldgaard et al., 2003; Leclerc et al., 2008). Although we did not analyze samples from unfragmentated headwater populations, neither slope nor elevation was found to influence genetic structure, indicating that there is no bias against upstream dispersal besides of barriers. These variables were similarly inconsequential in other fish species such as *Catostomus catostomus* (Salisbury et al., 2016).

6.4.3 Bottleneck and effective population sizes

Evidence of ancient bottleneck was found in Cares 8, possible due to the construction of both an upstream dam in 1995 and a downstream dam in the 20s, which produce habitat fragmentation and decrease of gene flow locating this population between two impermeable barriers. Bottlenecks have been associated with fragmentation in previous studies (e.g. Coleman et al., 2018) and can contribute to genetic diversity loss and increases in population differentiation. Historical bottleneck was only evident in Deva6, but its cause cannot be determined in the absence of further historical information. All the populations (except Casano2 and Cares2) showed adjusted N_e values over 100. This is the limit which is required to avoid inbreeding depression (Frankham et al., 2014). Furthermore, in the case of Cares2 this value was also higher than 1,000, which is the recommended limit to maintain evolutionary potential (Frankham et al., 2014). Populations with small effective population size are expected to reach intense genetic

drift and suffer higher probability of population extinction (Newman & Pilson, 1997), in addition to increase likelihood of fixation of deleterious alleles and reduced selection effectiveness (Hare et al., 2011). Casano2 showed infinite N_e estimates values possibly due to sampling error and a larger sample size might result in more reliable estimates (Waples & Do, 2010). The estimated ratio between effective and census size in salmonids is around 0.1–0.2 (Campos et al., 2006), but higher values have been also reported (Ardren & Kapuscinski, 2003). This value is important for monitoring changes in genetic diversity and predicting the rate of genetic loss (Ardren & Kapuscinski, 2003). In our study, this ratio presents relatively high values in some sites like Deva 6 and Cares 2. One possible explanation is the difficulty of sampling these sites leading to underestimation of their census sizes. On the other hand, the difficulty of delimiting a population in this scenario means that the area used to estimate census size could have been insufficient. In some studies, N_c is obtained extrapolating to the length of stream without barriers (Ruzzante et al., 2016) or extrapolating to occupied stream length (Peacock & Dochtermann, 2012) but there is no universal method. This lack of consensus highlights the need for further research on the definition and delimitation of populations for census size estimation for mobile organisms that inhabit river networks.

6.4.4 Conservation strategies and management implications

The obtained results have strong implications for management and conservation strategies of native brown trout populations. Identified genetically distinct populations could be recognized as genetically independent management units in the Deva-Cares catchment. Moreover, it is possible that a considerable number of distinctive genetic populations are yet to be recognized due to the presence of several barriers in other tributaries: a more detailed genetic study (i.e. expanding the river network to other headstreams) could identify additional distinct populations.

This work provides strong support for the critical role of connectivity and gene flow on the persistence of populations, securing their genetic diversity for the future. To achieve that goal, the ideal option would be to rewild the ecosystem (by total removal of anthropogenic barriers), but due to the high number of anthropogenic barriers present in the basin, nowadays it is a very unfeasible option. For this reason, it would be appropriate to identify key populations to conserve and key areas where to increase the genetic flow by removing barriers or increasing efficiency of fish passage. For those

populations in which connectivity cannot be improved, the habitat should be enhanced considering its positive relation with genetic diversity and genetic differentiation (Whiteley et al., 2013). In our study, Deva 11 contains a mixed genetic pool and constitutes an important genetic reservoir that should be considered as a priority area to conserve. In addition, isolated population susceptible to low levels of genetic diversity that provide downstream gen-flow to other populations, should be given full consideration in conservation efforts (Kelson et al., 2015). In our catchment, these populations are Casano2, Duje y Salvoron and all of them are above natural impermeable barriers. There are two main possible causes for the presence of fish populations above natural impermeable barriers: human transfer of fish to fishless streams (Rahel, 2007) or geological processes (Currens et al., 1990). Nowadays, there is no knowledge about the origin of brown trout above natural barriers in the Deva-Cares catchment. Future in-depth research on these populations could reveal if these rivers have been previously fishless or not to find the best conservation strategy. In the case of finding evidence of human transfer, should these populations be protected? Or should the stream return to be fishless? Currently with our study we cannot answer these questions indicating that for an appropriate management and conservation future studies will be necessary.

6.5 Conclusions

In conclusion, this study suggests that impermeable barriers have a large effect on the genetic variation of the native brown trout population inhabiting the Deva-Cares catchment, demonstrating significant differentiation between populations above and below barriers, together with an isolation by distance pattern. The presented analysis at a river network scale has provided evidence for the role of barriers in influencing patterns of genetic diversity, highlighting the importance of maintaining and restoring the connectivity of freshwater ecosystems for conserving diverse brown trout populations. Such results suggest that measures of management and conservation should be taken for longer-term viability of populations inhabiting the Deva-Cares catchment.

6.6 Supplementary material 6

Supplementary material 6 contains the Table S6.1 referred to in the main text of the Chapter VI.

Table S6.1. Microsatellite loci including locus name, repeat motif, respective multiplex group and fluorescent dye as well as the source reference used in the characterization of the genetic diversity of brown trout populations in the Deva-Cares catchment.

Locus	Motif	Multiplex reaction	Dye	References
Str15	$(CT)_n$	M1	FAM	Estoup *et al.* 1993
Str60	$(GT)_n$	M1	FAM	Estoup *et al.* 1993
Str73	$(GT)_n$	M1	FAM	Estoup *et al.* 1993
Ssa85	$(GT)_n$	M1	HEX	O'Reilly *et al.* 1996
Sssp1605	$(GATA)_n$	M1	HEX	Paterson *et al*. 2004
Ssa197	$(GTGA)_nGT$	M2	FAM	O'Reilly *et al.* 1996
SSOSL85	$(GT)_n$	M2	HEX	Slettan *et al.* 1995
SSOSL311	$(TG)_n$	M2	HEX	Slettan *et al.* 1995
SS4	$(GT)_n$	M2	FAM	Martínez *et al.* 1999
SSOSL417	$(GT)_n$	M3	FAM	Slettan *et al.* 1995
SSOSL438	$(GT)_n$	M3	FAM	Slettan *et al.* 1996
BFRO002	$(GT)_n$	M3	HEX	Susnik *et al.* 1997

Chapter VII

General conclusions and future research

Chapter VII: General conclusions and future research

7.1 General conclusions

Both the spatial connectivity and environmental variables (i.e. connectivity-dispersal versus niche concepts) have shown to be important on determining the spatial patterns of brown trout in the Deva-Cares catchment. The analysis and modelling approaches at river network scale developed in this PhD Thesis provide importance evidences on which are the main mechanisms defining the spatial patterns of brown trout in the Deva-Cares river network, what could be taken as an important example for other river networks.

The brown trout populations in the Deva-Cares catchment are spatially age-structured and the different dispersal capacities and niche preferences determine their spatial patterns, which are highly influenced by the network structure its connectivity and the different suitability of its river reaches for the different age-classes. The spatial patterns of brown trout (densities and genetic) and the modeled results are key to determine that the brown trout in the Deva-Cares catchment may be functioning as a metapopulation. A number of reasons support this metapopulation view: (1) the spatial variability of environmental variables throughout the river network generate discrete and different quality patches; (2) barriers and the spatial connectivity of the network cause the existence of empty and occupied patches; (3) the different dispersal abilities of the different age-classes between habitat patches. The dispersal ratio and the variation in patch size or quality evidence that the metapopulation type may be more close to a mainland-island or source-sink structure. However, a significant pattern of isolation by distance was also found in the brown trout population in the Deva-Cares catchment involving that both model, metapopulation and member-vagrant, are present in the catchment and different part of the network could present different behaviour.

Spatial patterns and population dynamics of brown trout population in the Deva-Cares catchment are highly affected by habitat fragmentation and loss of connectivity due to the presence of longitudinal impermeable barriers. This alteration modifies their natural spatial patterns and might influence the persistence of brown trout population in the

Deva-Cares catchment evidencing that management, planning and conservation measures should be taken into account at the river network scale.

In chapters III, IV, V and VI of this PhD Thesis, we investigated the determination and modelling of spatial patterns of brown trout in the Deva-Cares catchment through the role of the connectivity and the niche at the river network scale. The conclusions obtained in this PhD Thesis provide scientists and managers an important insight over the spatial patterns of brown trout at a river network scale and will assist in the management, planning and conservation of the species.

Following, general conclusions are presented for each of the thesis chapters:

Chapter III. Mapping the temporary and perennial character of whole river networks

- The proposed approach for estimating the occurrence and extent of perennial and temporary segments can be applied to any other river network in the world considering the applicability of our assumptions. Information on the temporal-perennial character for a whole river network is usually not available, incomplete, or not very precise. Thus, with information relatively easy to collect and using minimal data resources (field data, access to aerial images and a virtual watershed approach) it is possibly to build a temporal and perennial river segment classification model for whole river networks.

- The incorporation of available knowledge from locals and experts represents an improvement in the mapping approach and in the final digital maps.

- Catchment area, area occupied by broadleaf forest, minimum monthly precipitation in August and average catchment elevation are the most important catchment characteristics that play a fundamental role in determining the spatial distribution of flow permanence at catchment scale.

- The length of perennial river segments is considered the available habitat for brown trout populations. However, the length of temporary river segments represents a highly percentage of the total channel length of the river network. In the Deva-Cares catchment most of these temporary channels were in the higher parts of the network, thus they were not that important for controlling river reach connectivity for fishes. However, temporary streams should not be neglected in

other catchment scale studies in order to increase our understanding of how hydrologic variability and the spatial connectivity that intermittency might produce for riverine communities.

Chapter IV. Spatial variability of Salmo trutta at a river network scale. What variables are influencing spatial distribution of population density?

- Brown trout population density is spatially age-structured and niche and dispersal are both important factors influencing the spatial variability of brown trout density at the river network scale. The importance of niche and dispersal principles change depending on the age-class considered. The higher mobility of older age-classes increases the importance of the dispersal-connectivity relation, while niche characteristics are more important in earlier life stages with less mobility.

- Importance of environmental variables at different spatial scales differed among age-classes. Environmental variables at catchment, segment and reach scale are more important for young-of-the-year and adults, showing the young-of-the-year class a dependency on adult density, while the juvenile class is more influenced by river reach variables.

- Connectivity mainly determines the presence/absence of the species, while the environmental variables influences more the carrying capacity or average densities of the species in each river reach (or patch). Both factors should be considered together in order to better understand spatial patterns on trout densities.

Chapter V. Effects of altered river network connectivity in the distribution of Salmo trutta: insights from a metapopulation model

- The proposed numerical metapopulation model is an appropriate tool to estimate the average spatial patterns of age-specific brown trout density in a whole river network and to assess the impact of altered connectivity.

- The metapopulation model based on topology, connectivity, population dynamics and dispersal characteristics show that spatial heterogeneity in brown

trout density is highly dependent on connectivity and population dispersal characteristics such as adult dispersal rate and direction of movement.

- Altering the natural connectivity of a river network results in major changes in the spatial distribution of brown trout density. Removing a single obstacle might have consequences on fish density even in distant tributaries, while removing all longitudinal barriers to fish migration in the river network will restore brown trout density levels to a more natural pattern.

Chapter VI. Genetic evidences and consequences of river network connectivity on a native Salmo trutta population

- Impermeable barriers have a large effect on the genetic variation of the native brown trout inhabiting the Deva-Cares catchment acting as an isolation mechanism. Below-barrier populations showed high levels of genetic diversity and lower values of genetic differentiation, while headwater and above-barrier populations showed low levels of genetic diversity and high values of genetic differentiation. Isolation by impermeable barriers played a more important role than hydrological distance on determining the genetic structure of the populations, although an isolation by distance pattern also exists in the Deva-Cares catchment, probably caused by low distances travelled by migrant individuals.

- A decrease in genetic variability in upstream sites and a downstream-biased gene flow is possibly caused by fragmentation and the consequent transport downstream of some individuals by flow, amplifying the dispersal asymmetry, since the results from historical migration indicate that gene flow between all pairwise comparisons was symmetric.

- Most of the populations showed small effective population size, which could lead to an intense genetic drift and higher probability of population extinction.

- Persistence of populations depends critically on connectivity and gene flow. Measures of management and conservation should be taken for longer-term viability of populations inhabiting the Deva-Cares. Population of Deva 11 is a priority area to conserve as it contains a mixed genetic pool and constitute an important genetic reservoir. In addition, Casano2, Duje and Salvorón

populations should be given full consideration in conservation efforts because are susceptible to low levels of genetic diversity and provide downstream gen-flow to other populations.

7.2 Future research

According to the objectives established in this PhD Thesis, we identified important issues determining the spatial patterns of brown trout at river network scale, which provide important knowledge necessary to be taken into account in effective management, planning and conservation strategies. In addition, this PhD Thesis also revealed the existence of certain knowledge gaps and new research questions that should drive future research. Some of the most relevant aspects that require future research are outlined below.

- The results obtained in this thesis showed a major improvement of the digital cartography of temporary and perennial river channels in the Deva-Cares catchment highlighting the need to update the current information of the digital cartography of temporary and perennial river channels in other areas. Incorporation of other variables or methods to obtain dependent and independent data should be analyzed in order to apply this methodology in catchments where different hydrological processes dominate and our initial assumptions are not met.

- Incorporation of information about frequency and duration of temporary flow and presence of flow discontinuities (e.g. sinkholes) should be considered in future research in order to obtain dynamic maps that provide more information to delimit the available habitat for brown trout populations.

- The different results obtained in the GLM adjustment in chapter IV considering locations where the species is present and absent or only considering locations where the species is present showed that it is necessary to carry out future studies considering the application of different initial data and evaluate what the effect on the results is. Moreover, it is needed to account for possible different results applying our methodology on different fish population characteristics such as biomass instead of density. These future results will also contribute to design efficient and appropriate field campaigns to analyze and model data at river network scale (e.g. balanced data with equal number of presences and absence sampling points).

- Future improvements to the numerical metapopulation model developed in the PhD Thesis should take into account the spatial-temporal heterogeneity of the population dynamic variables. Specific empirical studies should be conducted to obtain optimal spatio-temporal data of the model parameters according to the local populations in the study area. Population dynamic variables that need to be specially investigated by the absence of data are those related to the movement and dispersion of the species (e.g. distance and direction of movement tracking for each age-class and the proportion of sedentary/mobile individuals).

- Temporal fluctuations of environmental variables are also important in determining changes in fish densities, which were not taken into account into our study. The numerical metapopulation model should be enhanced by including temporality and stochasticity. This future approach would allow analyzing how situations or scenarios not contemplated in this PhD Thesis (e.g. climate change or hydrological alterations) might affect spatiotemporal patterns of population dynamics.

- Temporal genetic studies, in addition to the spatial genetic approach used in this PhD Thesis, should also be conducted to identify a particular evolutionary model of brown trout in the Deva-Cares catchment.

- An analysis of recent migration by genetic analysis is also necessary to investigate in depth the gene flow in the Deva-Cares network. Future research on the species movement commented previously in conjunction with these analyzes will provide a detailed knowledge of species dispersal at river network scale. We consider that the availability of this information is critical to establish limits of population composed of mobile organism and to estimate population census.

- The existence of several genetically distinct populations in the Deva-Cares catchment has been shown, thus, these results suggest that there could be more genetic units in the catchment due to the existence of barriers and populations of trout in other parts of the network not analyzed. A more detailed genetic study should be conducted to identify additional distinct populations.

- The current knowledge about the origin of brown trout populations above natural barriers in the Deva-Cares is null. A future study to define if these rivers have been previously fishless or not is necessary to apply an appropriate management and conservation strategy to those subpopulations and for the wider brown trout metapopulation.

Annex

Preliminary study

Annex: Preliminary study

Integration of habitat models to predict fish distributions in several watersheds of Northern Spain

This annex is an edited version of the research article published in the Journal of Applied Ichthyology, 32, 204-216, by González-Ferreras, A.M., Barquín, J. and Peñas, F.J. in 2016 with the title "Integration of habitat models to predict fish distributions in several watersheds of Northern Spain". doi: 10.1111/jai.13024

Abstract

Species distribution models and consensus models allow knowing the distribution of species in large areas where no field data exists and identifying the most important drivers for those distributions. In this study, seven individual models were used to obtain a consensus model to determine the potential distribution for six freshwater fish species in several watersheds of Northern Spain. Moreover, three different methods of model evaluation were used for performance comparison. Fish data were obtained from databases provided by different organisms related to aquatic systems containing information on 759 field sites sampled between October 2002 and June 2011 using electrofishing techniques. Dependent variables were obtained after filtering field sites according to a human pressure gradient analysis, while independent variables were derived from a Synthetic River Network for the study area. The "best" individual models were obtained using Random Forest, Generalized Boosted Models and Generalized Additive Models, but with different results among species and evaluation methods. The different consensus models revealed a high degree of adjustment between modelled and observed data. The most important factors related to fish distributions were the width of the valley floor, mean annual flow, average catchment elevation, distance to the sea and total catchment area. The importance and the critical limits of presence-absence for these key variables differed among species. Use of these models could assist in the prioritization and selection of specific catchment and river reach actions for fish population management, restoration and/or conservation.

1. Introduction

Aquatic ecosystems are among the most diverse on earth. Gradients in salinity, temperature, availability of light, dissolved gases and nutrients, along with biogeographic processes have all contributed to the diversity of biological communities and species in these ecosystems (Geist, 2011). While marine communities contain more diversity, freshwaters are far richer per unit habitat volume (Ormerod, 2003) and estimated to represent nearly 10% of the total number of animal species globally (E. V. Balian et al., 2008). As in terrestrial ecosystems, knowledge of the number of species inhabiting freshwater aquatic ecosystems is more complete for vertebrates than for invertebrate species. Freshwater fish are the most diverse of all vertebrate freshwater groups, with nearly 10,000 described species, but they are also the most highly threatened (Duncan & Lockwood, 2001).

The freshwater fish fauna of the Iberian Peninsula shows a distinctive position within the European ichthyofauna (Corbacho & Sanchez, 2001). They are characterized by having a large number of endemic species. Nevertheless, they have a low regional diversity compared to other areas in Europe (Carmona et al., 1999). This is mainly attributed to different historical environmental events, the location of the Iberian Peninsula, as well as the presence of biogeographical barriers (e.g. Pyrenees). The Iberian fish fauna is also among the most endangered within the European continent, as the catchments of the largest Iberian rivers have been largely modified in relation to hydrological and habitat characteristics (Tockner et al., 2009). At the present time there is an urgent need for an adequate management of aquatic populations. In the particular case of fish populations, some Iberian species are included in European and National legislation as the Habitats Directive (43/92 C.E.E.) or on the list of freshwater fish species within the Red book of Spanish Vertebrates (J. C. Blanco & González, 1992). In addition, recent studies have shown noticeable reductions in Iberian Peninsula fish populations by such causes as climate change (e.g. Almodovar et al., 2012). Thus, there is an urgent need to improve our knowledge on their potential distribution and the factors that control this on a large spatial scale.

A comprehensive assessment of fish biodiversity and the fish community conservation status and their possible relationship with environmental variables and river alterations are key aspects to establishing a proper conservation management plan (Clavero et al.,

2004). In relation to this, species distribution models (SDMs) are gaining importance as useful tools for fish management or to explore diverse questions in ecology, conservation and evolution. SDMs are defined according to Benito de Pando (2009) as a numerical construction, which defines the ecological relationships between the presence of species and the values of environmental variables influencing their distribution. Results from SDMs are expressed in geographic space as a digital map showing the suitability of the habitat or the probability of species occurrence. SDMs have been applied in studies of climatic change (Lyons et al., 2010), invasive species (A. T. Peterson, 2003), aquaculture (Perez et al., 2003) and conservation of endangered species (Benito de Pando & Peñas de Giles, 2007) such as freshwater fish (Leathwick et al., 2006), marine fish (Hedger et al., 2004), plants (Bedia et al., 2011), birds (S. Manel et al., 1999) or reptiles and amphibians (Segurado & Araujo, 2004) among other groups of animals.

Although these methods are numerous and commonplace in the scientific literature, much debate centres on which statistical modelling approach is most appropriate for predicting species distributions (Hoffman et al., 2010) because a modelling technique that works well for a given species or modelling problem is not necessarily appropriate for others (Kampichler et al., 2010). Currently, there is a new approach where sets of techniques are used to obtain consensus models, seeking to reduce the biases and limitations of the individual use of just one modelling technique (Pliscoff & Fuentes-Castillo, 2011). This approach is based on the idea that different predictions are copies of possible states of the real distributions, and that all of them form an ensemble (Marmion et al., 2009).

The present study aims to (1) perform seven individual models to predict the potential distribution of six different autochthonous fish species that are important from an ecological, social and economic perspective in the Iberian Peninsula and compare their results with three different methods of evaluation. These species are *Anguilla anguilla* (Linnaeus, 1758), *Barbus haasi*, (Mertens, 1925), *Luciobarbus graellsii* (Steindachner, 1866), *Parachondrostoma miegii* (Steindachner, 1866), *Salmo salar* (Linnaeus, 1758) and *Salmo trutta* (Linnaeus, 1758); (2) elaborate a consensus model for each species from the "best" single models obtained from the first objective; (3) obtain suitability maps for each species under nearly natural conditions and the relative importance of the key independent variables determining their potential distribution.

2. Methods

2.1. Study area

The study area is located in Northern Spain (Figure A.1). It is delimited by the Cantabric Sea in the north and the Mediterranean Sea in the east. It represents heterogeneous environmental conditions and it has a wide variety of bioclimatic areas, because of the confluence of the Mediterranean and Temperate macroclimate and the altitudinal gradient imposed by the many mountain ranges (CHC, 2019). It can be broadly segregated in two main zones. On the one hand, the area draining into the Cantabric Sea encompasses several small basins with drainage areas ranging from 30 km^2 to 4.907 km^2 covering a total area of 22000 km^2. Rivers are confined by the Cantabrian Cordillera, a mountain range that runs parallel to the coast and reaches up to 2600 m.a.s.l. Hence, they are characterized by high slopes and short river lengths. This area has a Temperate climate (CHC, 2019). Average annual temperature is 14 ºC and precipitation is abundant throughout the year with mean of 1300 mm $year^{-1}$, presenting maximum rainfalls in December (150 mm $month^{-1}$) and minimum in July (50 mm $month^{-1}$). However, the precipitation magnitude and distribution varies significantly according to local topography. Snow precipitation is frequent in winter above 1000 m.a.s.l. Population density is 175 inh. km^{-2} although it is concentrated especially in cities nearly to the coast and in wide valleys. On the other hand, the Mediterranean area is mainly occupied by the Ebro basin. It covers a total extension of 85530 km^2. This catchment is enclosed by the Cantabrian Mountains and the Pyrenees (3400 m.a.s.l.) in the north, by the Catalan Coastal Chain (1712 m.a.s.l.) in the east and from the north-west to the south-east by the Iberian Massif (2300 m.a.s.l.) which creates a dense river network in the catchment boundaries and an extended flat surface in the interior. It receives both Temperate and Mediterranean climate influences. Annual precipitation is 656 mm, however it varies significantly from 300 mm in the centre to 1700 mm in the highest mountains (Bejarano et al., 2010) where snow is also common during the winter. The temperature regime also presents oscillations throughout the year with temperatures over 30 ºC in summer and below 5 ºC during winter. Population density is below 35 inh. km^{-2}.

In relation to vegetation, the area is divided into two main regions: Eurosiberian and Mediterranean. Mediterranean vegetation is dominated by evergreen sclerophyllous

species while Eurosiberian vegetation is dominated by deciduous forest. There are alpine mountain grassland and denuded rocks at higher altitudes. In extensive areas, the natural vegetation has been highly modified by human actions and we find pasture sections across the whole study area and eucalyptus plantations (*Eucalyptus globulus*) in the northern coastal area, while agriculture dominates within the Ebro depression.

Finally, the study area is mainly formed by gravels, sands, silts, conglomerates, sandstones, clays and limestones in the Ebro basin, while the Cantabrian area is represented mainly by marls, dolomites, limestones, sandstones, shales and conglomerates (IGME, 2015).

2.2. Fish data

Species selected for this study are identified under study objectives at the end of the introduction. Fish data were obtained from existent databases (biomass and abundance) within the Environmental Hydraulics Institute "IH Cantabria", Government of Cantabria, Basque Water Agency (URA), Catalan Water Agency (ACA), Ebro Hydrographic Confederation (CHE) and Cantabric Hydrographic Confederation (CHC). These databases contained information from 759 field sites that were sampled between October 2002 and June 2011 using electrofishing techniques. These surveys were carried out by different personal and with different periodicity, in some cases using a single capture and in other cases using successive captures. The sampling area varies from 21.6 m^2 to 4400 m^2, depending on river size and type. We are aware that this database is very heterogeneous, but its use allows maximizing the spatial coverage of field distribution data for the selected fish species, as it has been done in previous studies (e.g. Leathwick et al., 2005).

With the intention of predicting fish species potential distribution, we selected field sites that were considered to be under nearly natural conditions or with minimum human impacts for our modelling data set. To accomplish this, we performed an analysis of pressures using geographical information for each field site. All geographical analysis were carried out with ArcGIS Desktop 10 ® Education Edition software (ESRI, 2011). Main threats that alter the functioning and connectivity of Iberian rivers are related with habitat degradation, hydrological alterations and exotic species (see Maceda-Veiga, 2013 for an overview of the main threats). These threats or the interaction of many

threats affect the fitness of native fish populations. In this study, we considered five main categories of human pressures to perform a hierarchical analysis of pressures. We included by order of importance: (i) land cover, (ii) hydrological alterations, (iii) connectivity alterations, (iv) point source discharges and (v) non-native species. Land cover data were derived from CORINE Land Cover 2006 while the rest of pressures data were provided by the same organizations cited above from existing databases with data collected until 2011 or available data closest to 2011. All information was integrated and organized in different GIS thematic maps.

Spanish river ecosystems are subjected to a high degree of anthropogenic disturbance (Prenda et al., 2006). For this reason, we could not strictly apply a total absence of these types of pressures from our dataset selection, because the final number of locations would be too low to generate any SDM. Therefore, in order to retain a minimum number of field sites within our modelling data set we established thresholds for each pressure (Table A.1). The process followed a hierarchical approach in which we applied first the land cover criteria to all the available field sites and then the hydrological criteria and so on. The last criterion applied was the non-native species presence.

The SDMs that we developed were all based on presence-absence (P/A) data. Because of the heterogeneity in our original database (covering different years and seasons) we performed an analysis on P/A data previous to the selection of the modelling data set. We checked that P/A data were homogenous among years and seasons when we had more than one sampling occasion per field site. Identical P/A records were observed in more than 95% of these cases. In the remaining sites (only 5% of the field sites), we followed the below criteria to extract fish P/A data:

In field sites with multiple season data we selected summer sampling occasions, as summer was the most represented sampling occasion. In field sites with multiple year data, we selected data from the more "normal" hydrological year (according to the data of MMARM, 2011). Additionally, when there was just data from a "wet" or a "dry" year in the same site, we chose the wet year because droughts produce a greater negative effect on fish than floods (e.g. Humphries & Baldwin, 2003).

After this data selection we obtained a total of 193 field sites under nearly natural conditions (Figure A.1). The frequencies of occurrence for each of the selected species

were 40.9% (*A. anguilla)*, 8.8% (*B. haasi)*, 5.7% (*L. graellsii)*, 10.4% (*P. miegii)*, 19.2% (*S. salar)* and 94.3% (*S. trutta).*

Table A. 1. Type of human pressures used in the analysis of pressures to select fish field sites for the modelling data set. Fish data come from surveys conducted between October 2002-June 2011. Pressures data come from existing databases with the available data closest to 2011, except land cover data (derived from CORINE Land Cover 2006). Criteria and number of deleted sites are shown at each stage

LAND COVER	
Measure	Area occupied by agricultural or urban uses upstream the river reach or in a 200 m buffer along the length of the surveyed river reach.
Altered Sites (415)	River reaches with more than 10% of urban and agricultural uses in the upstream catchment or more than 40% in a 200 m buffer along the length of the surveyed river reach (Atlantic rivers). River reaches with more than 10% of urban and agricultural uses in the upstream catchment or more than 70% in a 200 m buffer along the length of the surveyed river reach (Mediterranean rivers).
HYDROLOGICAL ALTERATION	
Measure	Dams with height ≥10m or without height data.
Altered Sites (56)	Field sites placed in the downstream segment of a dam not having any upstream tributary between the dam and the field site location of equal or higher river order.
CONNECTIVITY ALTERATIONS	
Measure	Dams and weirs with height >0.5m or without height data.
Altered Sites (38)	Field sites located in river reaches upstream or downstream a dam or a weir being the length of naturally connected river channel ≤5000 m
POINT SOURCE DISCHARGE	
Measure	Industrial, urban or assimilated point discharges (≥2000 p.e.).
Altered Sites (55)	Field sites located at 5000 m downstream of a point discharge and not having any upstream tributary between the point discharge and the field site location of equal or higher river order or if this tributary has a point discharge in a distance ≤ 5000 m to its junction with the river reach under consideration.
PRESENCE OF NON-NATIVE SPECIES	
Measure	Presence of non-native species.
Altered Sites (2)	Field site whit presence of non-native species.

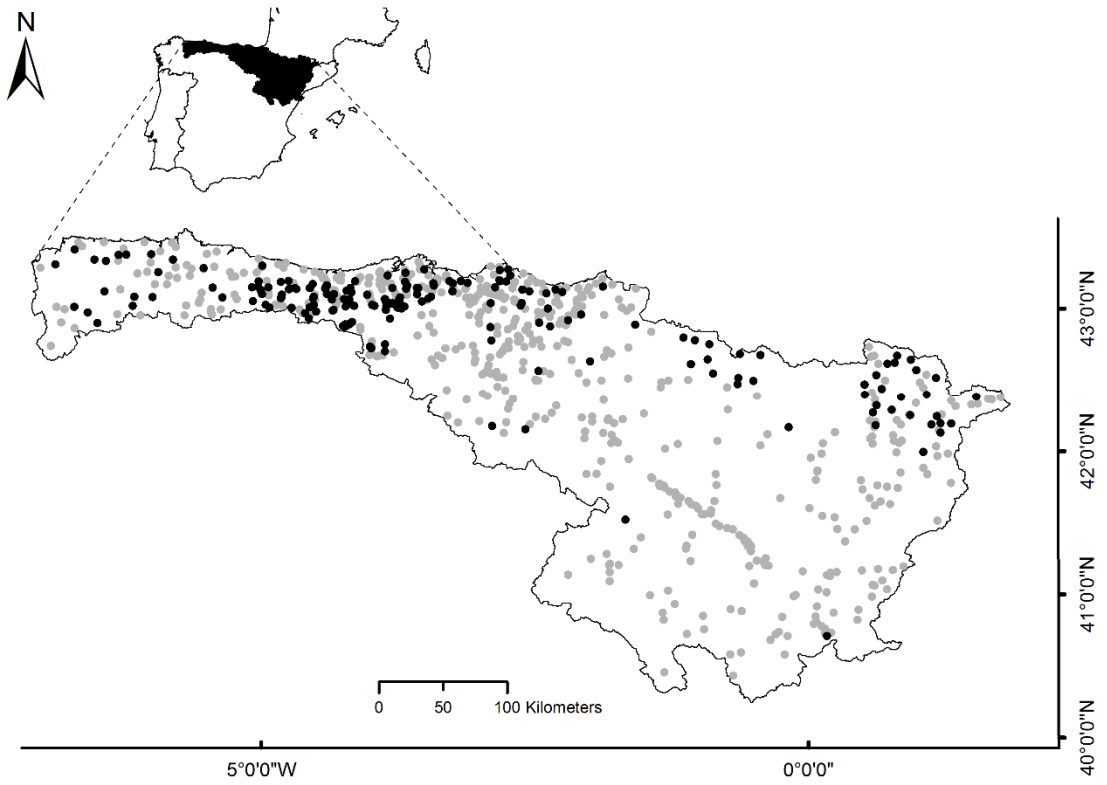

Figure A 1. Location of the study area and field sites. Black points (n=193) are sites considered under nearly natural conditions after the analysis of pressures, while grey points (n=566) show the location of sites dropped out in the analysis of pressures. Field sites data come from surveys conducted between October 2002 and June 2011.

2.3. Synthetic River Network and Independent Variables

In this study, Synthetic River Networks (SRNs) were delineated using flow directions inferred from a 25-m digital elevation model (DEM) using specific software packages (Buildgrids and Netrace) which are included in the 'NetMap' platform (Miller, 2002a; www.netmaptools.org). The river network was divided into reaches with lengths ranging from 16.9 to 823.6 m and was also divided in tributary confluences, as these can cause significant morphological changes in the channel and floodplain (Benda et al., 2004). The final SRN comprised 584628 river reaches and sets the spatial network to integrate the environmental information.

Variables in connection with topography, climate, hydrology, land cover and geology were hypothesised to be important on freshwater fish distribution regardless of geographic location. Independent variables describing several environmental attributes

including topography (n=9), climate (n=2), hydrologic (n=4), land cover (n=3) and geology (n=2) were extracted from existing databases provided by several national and regional organizations. The selection of these variables was based on previous ecological knowledge, that is, we selected the variables that a priori we thought they might be influential to the selected fish species distribution (Table A.2). Due to the influence of spatial scales in the distribution of fish species, we decided to include some of the variables at catchment level (MN), segment wings (i.e. sub-catchments; LC) or within a 200 m buffer along the whole length of the surveyed river reach (BF). The calculated variables used as independent variables in the SDMs are described below.

Table A. 2. Independent variables attributed to the Synthetic River Network in the initial set of variables. Bold variables are uncorrelated variables (Spearman rank correlation $< \left| 0.7 \right|$) included in the final set of predictor variables.

TYPE	CODE	DEFINITION	UNITS
Topographic	**AREA**	**Total catchment area**	**km^2**
	MN_ELEV	**Average catchment elevation from the considered river reach to the upper most river reach in the river network**	**m**
	BF_ELEV	Average 200 m buffer elevation	m
	MN_GRAD	**Average catchment gradient from the considered river reach to the upper most river reach in the river network**	**%**
	LC_GRAD	**Average segment gradient**	**%**
	BF_GRAD	Average 200 m buffer gradient	%
	ORDER	River reach order (Strahler)	1-9
	VAL_FLOOR	**Width of the valley floor at 5 x bankfull depth elevations above the channel**	**m**
	TO_OUTLET	**Distance from river reach to river mouth**	**m**
Climatic	MN_TEMP	Mean annual catchment temperature	ºC
	BF_TEMP	Mean annual segment 200 m buffer temperature	ºC
Hydrologic	**L1**	**Mean annual flow**	**m^3/s**
	MEAN7DAYFL	Mean of annual maximum 7 day flow	m^3/s
	MEAN_AUG	Mean daily flow for August	m^3/s
	FRE7	Mean number of events per year where flow is 7 x median flow	$yr.^{-1}$
Land cover	**BF_BFP**	**Area occupied by broadleaf forest within a 200 m buffer along the surveyed river reach**	**%**
	BF_CFP	**Area occupied by coniferous forest within a 200 m buffer along the surveyed river reach**	**%**
	LC_PAS	**Area occupied by pastures within the segment wings**	**%**

Table A 2. (Continued)

TYPE	CODE	DEFINITION	UNITS
Geological	**BF_HARD**	**Average rock hardness in a 200 m buffer along the surveyed river reach**	**1-5**
	MN_COND	**Average rock conductivity from the considered reach to the most upper catchment point in the catchment**	**1-5**

i) Topography: Catchment area, slope, elevation, order, distance from river reach to river mouth and valley floor width were derived from the 25-m DEM. In the case of valley floor width, we used valley width at a height of 5 times the bankfull depth elevation above the channel as an approximation of the real valley width (for more information see: Fernandez et al., 2012).

ii) Climate: Temperature was derived from monthly averages calculated in a 1 km grid map by means of interpolation procedure based on data recorded in more than 5000 weather stations of the Spanish network. These data were originally developed to be implemented into the Integrated System for Rainfall-Runoff modelling (in Spanish SIMPA model; Estrela & Quintas, 1996) by the Centre for Hydrographic Studies (CEDEX, Ministry of Public works and Ministry of Agriculture and Environment, Spain) for the assessment of water resources in natural regime at a national level.

iii) Hydrologic: We calculated four hydrologic variables referring to frequency and magnitude events (Table A.2; Hydrologic). These variables were derived using a set of functions from natural flow regime series (unaltered gauge) and predicted through Random Forest model (Breiman, 2001) for the whole study area (Peñas et al., 2014).

iv) Land cover: We achieved the percentage surface occupied by broadleaf forest, coniferous forest, scrubs and shrubs, pasture, agricultural land, denuded areas, wetlands and water and urban areas from the classification of land cover (CORINE Land Cover 2006). In turn, we obtained the percentage value for each class corresponding to the area occupied by a particular use in a 200 m buffer along the surveyed river reach in the upstream catchment of the river reach and within the segments wings. Due to the large amount of information available (8 land cover classes x 3 different spatial measures), we selected for the initial set of independent variables only the area occupied by pastures within the segment wings and the area occupied by broadleaf forest or coniferous forest within a 200 m buffer along the surveyed river reach (Table A.2; Land

cover). Other land cover variables (agricultural and urban areas) were taken into account in an indirect way in the analysis of pressures explained above.

v) Geology: The average rock hardness and conductivity were derived from the litostatigraphic and permeability map at scale 1:200000 developed by the Geological and Mining Institute of Spain. These variables were calculated using procedures described elsewhere (Snelder et al., 2008; Fernandez et al., 2012).

Finally, to avoid potential problems with multicolinearity among the 20 potential independent variables, we developed a correlation matrix (Spearman rank correlation) and when pairs of variables had a correlation $> |0.7|$ only one was retained for modelling. The final number of independent variables included in subsequent analysis was 12 (Table A.2).

2.4. Modeling

Models were constructed using the R 2.13.2 and R 2.14.0 software (R Development Core Team, 2011) and the editor Tinn-R 2.3.7.1 (Faria, 2011). To model species distributions, we used the "BIOMOD" package version 1.1-7.00 (Thuiller, 2011). BIOMOD requires P/A data of modelled entities and it allows combinations of several modelling techniques in an ensemble forecast (Thuiller, Lafourcade, Engler, et al., 2009).

2.4.1. Individual models

We used the next seven algorithms available in the BIOMOD package:

- MARS: Multivariate Adaptive Regression Splines (Friedman, 1991) is a non-parametric regression procedure that fits the response curve from segmented linear regression where the slope of the equation changes from one interval to another. Thus, the relationship between the dependent and independent variable is set based on a set of coefficients associated with the base functions determined from the data.

- RF: Random Forest is a technique developed by Breiman (Breiman, 2001) based on obtaining multiple decision trees. It generates several different random trees using subsets of observations and random subsets of the predictor variables,

where the predictions of the trees are performed by averaging in the case of regression trees or through a voting system in the case of trees classification.

- GLM: Generalized Linear Models (McCullagh & Nelder, 1989) are an extension of the linear models covering the main distributions of the exponential family. It decomposes the observed variability of a response variable in a systematic component and a random component linked together by a link function. It was selected by polynomial regression and a stepwise variable selection based on the Akaike information criterion (Akaike, 1973).

- GAM: Generalized Additive Model (Hastie & Tibshirani, 1990) are an extension of GLM. However, this approach considers all linear parametric effects, allowing expressing the effects using nonparametric smoothed functions. It was executed with four degrees of smoothing.

- GBM: Generalised Boosted Models (Ridgeway, 1999) is a non-parametric technique that employs a combination of two algorithms, regression trees and boosting, to generate and combine a collection of models. It aims to improve the performance of a single model by establishing many models for their subsequent combination. It was performed with a maximum of 3000 trees and 5 cross-validations to select the optimal number of trees.

- ANN: Artificial Neural Networks (Ripley, 1996) is a non-parametric machine learning model. In this case, the patterns of correlation between the independent variables and the response variable are identified through an iterative process. As different runs can provide different results, the best amount of weight decay and the number of units in the hidden layer were selected by using 3 cross-validations.

- SRE: Surface Range Envelope similar to BIOCLIM (McDonough et al., 2011). It is a model relating climate parameters with presence data to predict areas in which an organism can survive. It was performed using the quantil 0.05.

Distribution data for each species were partitioned randomly into calibration (80%) and evaluation (20%) datasets. Model accuracy was calculated using the area under the curve (AUC) of a receiver operating characteristic (ROC) curve (Fielding & Bell, 1997), Cohen's Kappa (Monserud & Leemans, 1992) and the True Skill Statistic (TSS;

Allouche et al., 2006). We implemented a cross-validation procedure to ensure that the final evaluation of the models is quasi-independent of the random data partition when the same data are used to construct and evaluate the model. The data splitting approach was replicated five times and it was the basis for calculating the mean AUC, TSS and Kappa of the cross-validations. These were considered to be the model accuracy values. To classify the accuracy of the models we followed Thuiller et al. (2009; Table A.3). Occurrence probabilities derived from all models (except SRE which provides P/A) were transformed into P/A data using the area under the ROC curve (AUC) and the threshold maximizing TSS and Kappa.

Table A. 3. Index value range for classifying model prediction accuracy according to the three evaluation methods.

MODEL ACCURACY	TSS	Kappa	AUC
Excellent or high	0.8-1	0.8-1	0.9-1
Good	0.6-0.8	0.6-0.8	0.8-0.9
Fair	0.4-0.6	0.4-0.6	0.7-0.8
Poor	0.2-0.4	0.2-0.4	0.6-0.7
Fail or null model	0-0.2	0-0.2	0.5-0.6

2.4.2. Consensus model

There are several different approaches to obtain a consensus model. The consensus model used in this study is based on a weighted mean probability, where weights are obtained from the TSS values of individual models. This approach has been recently proposed as one of the most robust (Marmion et al., 2009). We considered only evaluation on TSS because evaluation by AUC has been recently criticized (Wisz et al., 2008; Tognelli et al., 2009) and Kappa is sensible to prevalence (Allouche et al., 2006). Moreover, to avoid working with poorly calibrated models, only models with TSS>0.4 were considered because this value represents the minimum evaluation score for a model to be considered as valid. Single models were ranked according to their predictive performance, and a decay of 1.6 gave the relative importance of the weight, producing respective weights whose sum is equal to 1. Consensus model obtained for each species were used for predicting their distribution through the independent variable values for every reach of the SRN. Finally, to assess the fit of the consensus model we used Kappa, TSS and AUC values to compare the results obtained by the three methods.

2.4.3. Importance of independent variables and suitability maps

To estimate the variable importance and to assure comparability among single models, BIOMOD provides a permutation procedure to extract a measure of relative importance for each predictor variable. This procedure uses Pearson correlation between the standard predictions and the predictions where the considered variable has been randomly permutated. The importance of the variable for each model and each species was calculated as one minus the correlation between the standard prediction and the prediction where the considered variable was randomized (Thuiller, Lafourcade, Engler, et al., 2009). Moreover, we weighted the results obtained in this method with the same weight of each single model in the consensus model for each species and we calculated the percentage of relative importance to the consensus model.

Suitability maps or predicted geographic distributions were estimated based on consensus model projections for the whole study area. We elaborated suitability maps for each species with ArcGIS Desktop 10 ® Education Edition software (ESRI, 2011). These maps represented absence-presence probability calculated from threshold values obtained in the consensus model. The weighted probabilities of consensus model occurrence were converted in this format by a weighted mean threshold as they were ranked when using the TSS method. Probability values of presence were split into three categories (low, medium and high) with values equidistant from the threshold to the maximum probability value recorded for each species. Finally, based on the spatial distribution obtained and according to the relative importance of the independent variables, we analyzed the values of the variables with more importance for the zones in which it was predicted the presence of each species, because these variables are the most influential in their distribution.

3. Results

3.1. Individual models

In general, the individual models with the "best" accuracy were GBM, RF and GAM (Table A.4) according to the highest scores obtained through the three evaluation methods. RF was the model with higher AUC, TSS and Kappa scores for *S. salar* and *S. trutta*. The same model was also considered as the best for *P. miegii* (according to the

AUC method) and *B. haasi* (in accordance with AUC and Kappa methods). Meanwhile, GAM was chosen as one of the best method for *P. miegii* and *L. graellsii*, but only in line with TSS method (TSS=0.718, TSS=0.422 respectively). Moreover, in the case of *L. graellsii*, TSS score was the same for the GBM model, so in this case there are two models considered as the best for this species (TSS score). For *A. anguilla* the best predictive model was GBM following any of the evaluation methods. In the remaining cases (*B. Haasi*-TSS score*, L. Graellsii*-AUC and Kappa score *and P. miegii*-Kappa score) the best model was GBM. These best models were classified as fair, good or excellent except for *S. trutta* and *L. graellsii* which were categorized as poor when considering Kappa and AUC scores.

The "worst" method for almost all species was SRE (Table A.4) based on TSS and Kappa scores, except for *L. Graellsii* (Kappa) and *S. Trutta* (TSS), in those cases the worst models were ANN and GLM, respectively. In the case of AUC scores the models with lower values were different depending on the species, but in general they were ANN (*A. anguilla*, *P. Miegii*, and *S. salar*), GLM (*S. Trutta)* and MARS (*B. Haasi* and *L. Graellsii*). These worst models were categorized as null or poor, except for *A. anguilla*, *S. salar* and *B. haasi* where ANN and MARS had AUC>0.7.

3.2. Consensus model

The most frequently selected models to be included in the consensus model (TSS>0.4) were RF, GAM and GBM, whereas SRE was never selected. The number of models included varied among species. The minimum number was two for *S. trutta* and the maximum was six for *A.anguilla*, *B. haasi* and *S. salar*. The weights assigned to each model were different to each species in function of their TSS single model scores, but in general the models with higher weights were RF, GAM and GBM (Table A.5).

The consensus model fit with observed data was consistently high, with AUC, TSS and Kappa scores exceeding 0.9 for all species (Table A.5). In some cases as *B. haasi* and *S. trutta* these values were equal to 1 revealing a perfect fit to the observed data.

Table A. 4. Average values of TSS, Kappa and AUC in the final individual models. Bold values are considered the "best models" and underlined values are considered the "worst models". *Anguilla anguilla* 2 and *Salmo trutta* 2 values correspond to a re-analysis supporting some of the arguments in the discussion.

		ANN	GAM	GBM	GLM	MARS	RF	SRE
Anguilla anguilla	**TSS**	0.593	0.686	**0.720**	0.689	0.646	0.697	0.159
	Kappa	0.565	0.659	**0.714**	0.662	0.610	0.687	0.170
	AUC	0.808	0.901	**0.903**	0.867	0.846	0.901	--
Anguilla anguilla 2	**TSS**	0.665	0.749	**0.791**	0.778	0.679	0.785	0.281
	Kappa	0.658	0.743	0.783	0.772	0.669	**0.788**	0.287
	AUC	0.886	0.923	0.931	0.932	0.852	**0.941**	--
Barbus haasi	**TSS**	0.669	0.771	**0.943**	0.754	0.726	0.937	0.000
	Kappa	0.390	0.743	0.785	0.665	0.612	**0.809**	0.000
	AUC	0.836	0.907	0.968	0.876	0.759	**0.975**	--
Luciobarbus graellsii	**TSS**	0.228	**0.422**	**0.422**	0.217	0.194	0.411	0.072
	Kappa	0.047	0.200	**0.231**	0.082	0.128	0.152	0.103
	AUC	0.588	0.571	**0.640**	0.578	0.557	0.562	--
Parachondrostoma miegii	**TSS**	0.244	**0.718**	0.697	0.453	0.597	0.653	0.097
	Kappa	0.231	0.561	**0.640**	0.396	0.514	0.509	0.101
	AUC	0.635	0.850	0.850	0.749	0.695	**0.856**	--
Salmo salar	**TSS**	0.518	0.685	0.676	0.680	0.628	**0.714**	0.008
	Kappa	0.528	0.520	0.576	0.588	0.543	**0.605**	0.009
	AUC	0.776	0.851	0.864	0.841	0.849	**0.892**	--
Salmo trutta	**TSS**	0.267	0.478	0.283	0.239	0.256	**0.567**	0.311
	Kappa	0.292	0.161	0.092	0.060	0.060	**0.348**	0.057
	AUC	0.586	0.617	0.485	0.476	0.500	**0.671**	--
Salmo trutta 2	**TSS**	0.244	0.622	0.639	0.617	0.594	**0.706**	-0.150
	Kappa	0.244	0.622	0.639	0.617	0.594	**0.706**	-0.150
	AUC	0.622	0.864	0.873	0.849	0.837	**0.902**	--

Table A. 5. Weights of individual models used to elaborate the consensus model. Thresholds for the determination of species presence-absence in the consensus model and consensus model accuracy are also indicated. *Anguilla anguilla* 2 and *Salmo trutta* 2 values correspond to a re-analysis supporting some of the arguments in the discussion.

	WEIGHTS							TRES-HOLD	TSS	Kappa	AUC
	ANN	GAM	GBM	GLM	MARS	RF	SRE				
Anguilla anguilla	0.0380	0.0974	0.3988	0.1558	0.0608	0.2492	0	373.5695	0.9396	0.9356	0.9961
Anguilla anguilla 2	0.0380	0.0974	0.3988	0.1558	0.0608	0.2492	0	454.7320	0.9221	0.9236	0.9951
Barbus haasi	0.0380	0.1558	0.3988	0.0974	0.0608	0.2492	0	247.0355	1	1	1
Luciobarbus graellsii	0	0.4031	0.4031	0	0	0.1938	0	194.1291	0.9945	0.9538	0.9978
Parachondrostoma miegii	0	0.4145	0.2591	0.0633	0.1012	0.1619	0	156.7640	0.9827	0.9416	0.9983
Salmo salar	0.0380	0.2492	0.0974	0.1558	0.0608	0.3988	0	266.1068	0.9604	0.9451	0.9967
Salmo trutta	0	0.3846	0	0	0	0.6154	0	490.9808	1	1	1
Salmo trutta 2	0	0.1619	0.2591	0.1012	0.0633	0.4145	0	415.8136	0.9505	0.9505	0.9972

3.3. Importance of independent variables and suitability maps

To obtain suitability maps, we projected consensus model to the whole study area, except in the case of *S. salar*. For this species, we limited the projection area to only the Atlantic catchments as this species is only present in this area because of biogeographic reasons. There are other species in this situation (i.e. *B. haasi* is presents only in the Ebro basin) but it was not necessary to make this independent projection because the results obtained were coherent with their potential biogeographic distributions. The results obtained for each species are described below.

A. anguilla

The most important variables according to the consensus model were distance from segment to river mouth (ToOutlet_m) and average catchment elevation (MN_ELEV) with relative importance values of 68.1% and 16.1% respectively (Figure A.2A). The suitability map reflects the presence of *A. anguilla* in the northern river areas near the Atlantic Ocean and in the Ebro river mouth (Figure A.3A). The probability of occurrence is lower in more distant areas of the Atlantic Ocean and the Ebro mouth, as well as in the Spanish part of the Garonne catchment. The distance to the sea that marks

a limit on its presence is 180 km. In relation to altitude, they are distributed to almost 2300 m, corresponding to the Garonne river basin. Likewise, eel higher probability of occurrence happens at altitudes fewer than 1260 m and less than 40 km distance to the sea.

B. haasi

River reach distance to the sea was the variable that had a higher relative importance (57.6 %) for this fish species. The following most important variables were catchment altitude and average catchment gradient (MN_GRAD) with values of 14.5% and 10.2% respectively (Figure A.2B). Its distribution was associated exclusively to the Ebro basin (Figure A.3B). This fish is present in areas with distance to the sea ranging from 115 to 935 km and an average elevation from 200 to 3000 m. In relation to MN_GRAD, the minimum value was 0.01 % and the maximum was 2 %. The highest probability of presence occurs when distance to the sea ranges from 240 to 720 km, catchment altitude from 955 to 1905 m and catchment slope from 0.3 % to 0.7 %.

L. graellsii

The most important predictor variables for this fish species were distance to the sea, catchment altitude and valley floor width (VAL_FLOOR) with values close to 15% for all of them (Figure A.2C). The presence of this fish has been predicted for almost all the Ebro basin and some areas in the north belonging to the Atlantic basins (Figure A.3C). This fish species could be present in areas with a sea distance of up to 935 km. In connection with the other two predictor variables, the higher probability of occurrence is found in river reaches located at a range from 70 to 2000 m and with valley floor width from 30 to almost 9400 m.

P. miegii

The main predictor variables for this fish species were catchment elevation, distance to the sea and valley floor width, with relative importance values of 31.1%, 26.2% and 28.7%, respectively (Figure A.2D). *P. miegii* distribution under nearly natural conditions is located in specific areas of the north coast and in the largest part of the Ebro basin, but the higher probability of occurrence is clearly located in the Ebro depression (Figure A.3D). Its presence is predicted in areas with a maximum altitude of almost 3000 m, but the higher probability of occurrence is located at a maximum of

1890 m of altitude and in river reaches with less than 925 km distance to the sea and valley floor widths ranging from 70 to almost 9800 m.

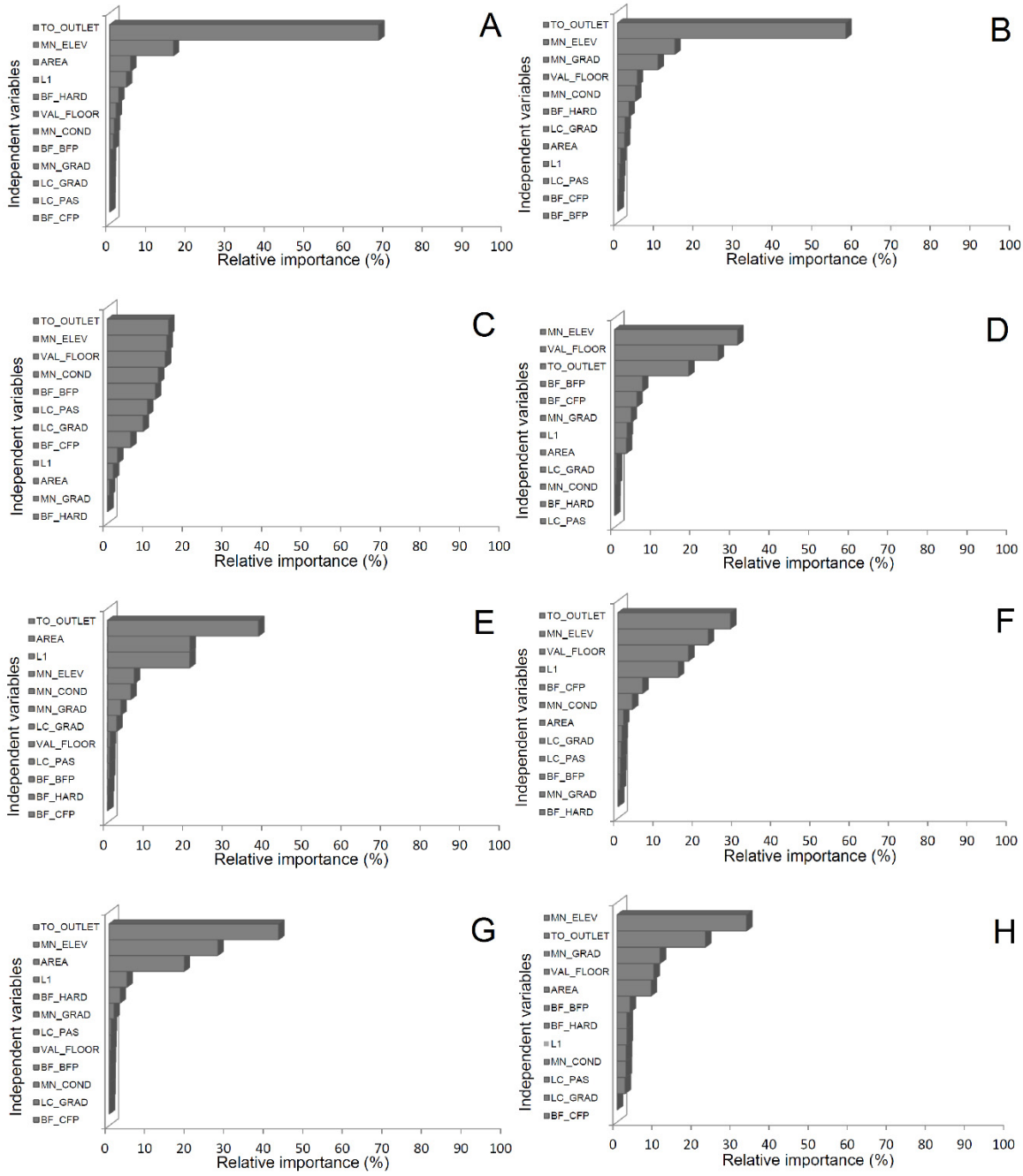

Figure A 2. Relative importance (%) of the independent variables (TO_OUTLET=Distance from river reach to river mouth, MN_ELEV=Average catchment elevation from the considered river reach to the upper most river reach in the river network, AREA=Total catchment area, L1=Mean annual flow, BF_HARD=Average rock hardness in a 200 m buffer along the surveyed river reach, VAL_FLOOR=Width of the valley floor at 5 x bankfull depth elevations above the channel, MN_COND=Average rock conductivity from the considered reach to the most upper catchment point in the catchment, BF_BFP=Area occupied by broadleaf forest

within a 200 m buffer along the surveyed river reach; MN_GRAD=Average catchment gradient from the considered river reach to the upper most river reach in the river network, LC_GRAD=Average segment gradient, LC_PAS=Area occupied by pastures within the segment wings, BF_CFP=Area occupied by coniferous forest within a 200 m buffer along the surveyed river reach) in the consensus model for each species (A=*Anguilla anguilla*, B=*Barbus haasi*, C=*Luciobarbus graellsii*, D=*Parachondrostoma miegii*, E=*Salmo salar*, F=*Salmo trutta*, G=*Anguilla anguilla* 2, H=*Salmo trutta* 2). Sub-figures G and H correspond to a re-analysis supporting some of the arguments in the discussion.

S. salar

The most important predictor variables determining the occurrence of this species were distance to the sea, catchment area and mean annual flow (L1), with 38.2%, 20.8% and 20.8 % relative importance, respectively (Figure A.2E). The high probability class for this species is represented by river reaches located less than 45 km to the sea (Figure A.3E), catchment area up to 1720 km^2 and mean annual flow ranging from 2.22 to 19.9 m^3/s.

S. trutta

This species suitability map shows that its distribution covers almost all river reaches within the study area (Figure A.3F). Its absence is associated mainly to areas near the estuaries and the lower probability of presence is associated with the northern boundary of the study area, the region near the Ebro mouth and the main channel of the Ebro River. The principal variables controlling trout distribution were distance to the sea, catchment elevation and valley floor width (Figure A.2F).

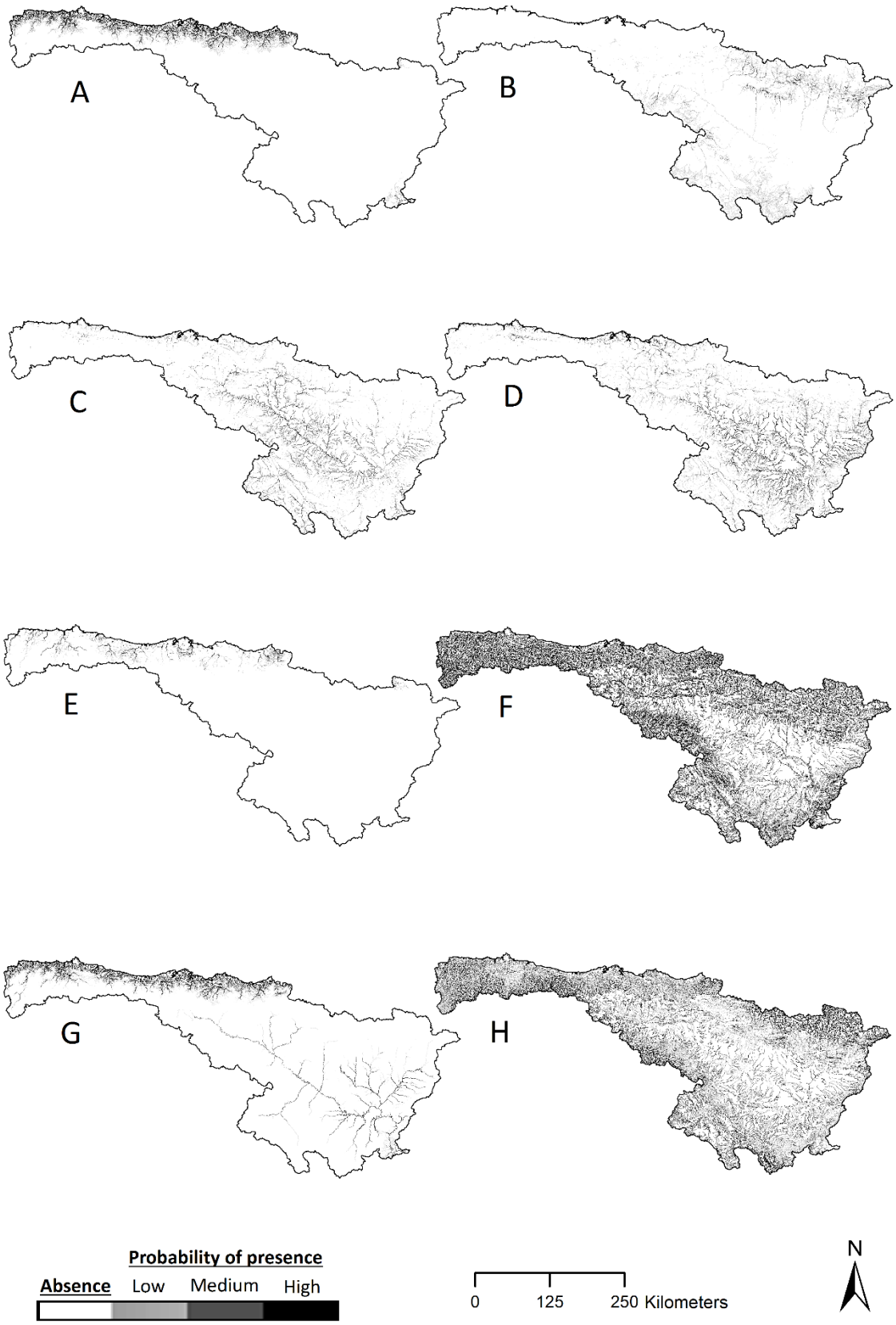

Figure A 3. Suitability maps of absence-presence probability obtained in the consensus model for each species (A=*Anguilla anguilla*, B=*Barbus haasi*, C=*Luciobarbus graellsii*, D=*Parachondrostoma miegii*, E=*Salmo salar*, F=*Salmo trutta*, G=*Anguilla anguilla 2*,

H=*Salmo trutta 2*). Field data used to model come from surveys conducted between October 2002 and June 2011. Sub-figures G and H correspond to a re-analysis supporting some of the arguments in the discussion.

4. Discussion

4.1. Individual models

In relation to the individual modelling results, RF, GBM and GAM exhibited greater predictive ability according to the three different statistics (area under the ROC curve, TSS and Kappa). However, there were notable differences among species contributing to the idea that there is not a best technique for all species, as it has been demonstrated previously by different authors (Thuiller, 2003). In general, *S. trutta* obtained the lowest predictive power regardless of model type and assessment methods. *S. trutta* is characterized by a wide distribution range in the study area, and then could be thought of as a generalist at this spatial scale. Specialist species tend to have much better predictive accuracy than generalists (Tsoar et al., 2007; Elith et al., 2008; Grenouillet et al., 2011). However, we believe that the main reason creating lower performance is the low number of trout absence data in our dataset since their frequency of occurrence is 94.3%. All models used need a suitable amount of P/A records in order to get accurate predictions (except SRE, in which predictions are only made with presence records). Thus, to test whether including field sites in the modelling dataset with absences improves model performance we have repeated the SDM for *S. trutta* but following a series of steps. First, we created a new modelling dataset which had the same number of field sites with presence and absence occurrence. To do that we randomly created false field sites with *S. trutta* absence data in the SRN by selecting river reaches that matched maximum and minimum independent variable values corresponding to field sites with real trout absence data (only 5% of the original dataset). This new modelling dataset comprised 364 field sites (50% presence and 50% absence) and the results of the new individual models (Table A.4; *S. trutta* 2) have considerably increased in accuracy. Moreover, in the case of *A. anguilla*, we detected an unequal distribution of records with presence and absence because is absent in the 55 field sites under nearly natural conditions in the Ebro basin. In order to explore the weight of this issue, we decided to redo the analysis including 18 field sites with presence data for the Ebro basin although they were not in natural conditions. In both species, model accuracy for the individual

models presented acceptable results for both the first and second modelling approaches, although a more balanced P/A dataset approach had slightly higher values for all models (Table A.4). Thus, it is advisable that the influence of the percentage of P/A data and the geographical cover of field sites in SDM performance and accuracy should be investigated in more depth using a combination of spatial scales and sets of generalist and specialist species. This will allow not only improving model statistics but also the digital representation of the actual and potential species distributions.

4.2. Consensus model

We established TSS metrics to include or not include models in the consensus model. In case that we would have chosen the area under the ROC curve or Kappa, the final consensus model would have been different. However, we considered that the choice of TSS is appropriate according to the given criteria in the methodology and our objectives.

Several authors have recommended the use of integration models as a way to increase the predictive ability of individual models (Thuiller, 2004; Grenouillet et al., 2011). In this case, the integration always yielded better fitted performances although consensus model predictive performance is still not easily calculated and this should be taking into account for evaluating predictions. In the case of the new models for *S. trutta* and *A. anguilla* the accuracy of the consensus model is slightly lower than the values of the first analysis, but in both cases is considered as a high score. The integration of models and consensus models are recent approaches in ecology (Araujo & New, 2007), so there is some debate about how to evaluate the accuracy (Fielding & Bell, 1997) when a SDMs or consensus model is used. In fact, we cannot yet establish a real comparison of the accuracy obtained from consensus model with the individual models. This is because in the consensus model we have used data to evaluate them that were used for its construction. Thus, the values of AUC, Kappa and TSS in the integration model only reveal the degree of fit. A more appropriated method to evaluate the accuracy of the consensus model can be the methodology followed by Marmion et al. (2009) who split calibration data set into two subsets ("inner-calibration" and "inner-validation"). Although it is highly recommended that evaluation data are independent of calibration data (R. G. Pearson, 2007), usually their availability is limited. Nevertheless, it is considered that the choice of a consensus model increases the accuracy of individual

models. Hence, consensus model do not always completely eliminates uncertainty, but is able to reduce the probability of making decisions based on predictions that depart from what can be considered the truth or reality (Araujo & New, 2007).

4.3. Importance of independent variables and suitability maps

In addition to evaluating the models from a statistical point of view, it is advisable to evaluate the results in relation to the spatial coherence and previous knowledge of the species ecological niche (Mateo et al., 2011). In some species there might appear confusing results in modelling due to biogeographic issues which are independent of model accuracy. In this case, to avoid problems with the prediction, *S. salar* was only projected for the Atlantic catchments. In future studies, we could consider the inclusion of some type of variable (e.g. geographical coordinates) for differentiating biogeographic issues within the modelling process and therefore avoid different projections for the same study area. For example, some studies have used sub-regions as categorical predictors of fish occurrences (Sindt et al., 2012).

Moreover, the critical limits and the importance of the independent variables could also be used to test how well the model represents the regional distribution of the selected species. In this study, the cases of *S. trutta* and *A. anguilla* illustrate this. Based on prior knowledge, there are some areas where their potential distribution in the first analysis is not well represented (Figure A.3A and A.3F), although the consensus models presented excellent results. Furthermore, when we performed the second modelling approach (see discussion of individual models) the values of the relative importance of the variables changed, but the most important variables remained the same (Figure A.2A, A.2F, A.2G, A.2H). In the case of *S. trutta*, the first obtained map showed a wide range of distribution with a high probability of presence in almost all the study area (Figure A.3F), while in the second one (Figure A.3H), we observe how the probability of presence has decreased especially in estuarine and low elevation areas. In the case of *A. anguilla*, we can see how it is hardly represented in the Ebro basin in the first obtained map, whilst the second approach shows an increase in the potential distribution area, especially in the Ebro basin and its major axis. Thus, for both species including a more balanced number of P/A sites according to the geographical context in which the species occurs determines that some critical limits of the predictors change to a more reasonable value. For example in the case of the presence of *A. anguilla*, distance to the sea

increases up to 840.5 km, while altitude decreases up to 1950 m. With respect to *S. trutta,* the changes of the values are less noticeable, but there is a large decrease in the number of river segments included in the probability class of occurrence when the second approach was used (Figure A.3F and A.3H). For both species, the second approach better represents their potential distribution and their ecological niche.

In connection with the independent variables, distance to sea, average catchment altitude, catchment area, average annual flow and valley floor width were the most important predictor variables for all the species, although importance varied among species. These variables have been previously shown as important determinants of fish distribution elsewhere, as in the case of *A. anguilla* (Domingos et al., 2006) or *S. trutta* (Joy & Death, 2004). Moreover, it would be interesting to include other chemical and physical variables that were not available for this study (e.g. the percentage of a certain type of mesohabitat could be important to simulate fish species richness; Olaya-Marin et al., 2013).

5. Conclusion

In conclusion, this study suggests that the use of consensus models is an appropriate tool to predict the distribution of fish species under nearly natural conditions. However, special care should be put on the selection of the modelling dataset, especially on the proportion of P/A data and on the spatial cover of the species distribution range. Moreover, both statistics and the limits of the predictor variables should be checked for model performance and accuracy. We consider that it is necessary a more detailed analysis that addresses all these issues in order to use distribution models adequately and to get a better protocol for drawing habitat suitability maps for a variety of species at regional scales. Finally, we have to remember that SDMs is a relatively new technique in ecology and it is in continuous progress and development, so it is expected that future applications might resolve some of the issues raised in this study.

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